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PRODUCTION OF SPANISH-TYPE GREEN OLIVES

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PRODUCTION OF SPANISH-TYPE GREEN OLIVES¹

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PRESENT WORLD CONDITIONS, with the prospect of foreign imports being curtailed and the canning of olives restricted in California, make it probable that more olives will have to be fermented or preserved by some method other than the canning process if the olives unsuitable for oil manufacture are to be utilized.

Although the canned California ripe olive has for some time been the major product of the olive industry in this state, the fermentation of green olives of the Spanish type has increased significantly during the past few years.

Increased production of the green fermented olive has been accompanied by many chemical and biological problems. If, in the present emergency, more olives are used for the production of this commodity it is anticipated that problems will arise in proportion to the number of processors now unfamiliar with the fermentation.

Investigations conducted since 1937 have increased our knowledge of the science and practice of green-olive fermentation. Since it is desirable that the new information be made available as well as integrated with earlier data, this bulletin has been prepared. The Sicilian type of pickled green olive is not covered in this publication.

THE INDUSTRY IN SPAIN

The Spanish-type green olive is identified by its green skin, light flesh, and light brownish-buff pit, when the pit is present. It has a characteristic flavor and aroma imparted by lactic acid fermentation; these in common with the inherent qualities of the fruit make it sought as an appetizing pickled olive in the United States, where it has found favor for many years. Fruit from any variety picked while immature and specially processed comes under this category; the Sevillano and Manzanillo varieties, however, are most often used. The olives may be whole, pitted, or pitted and stuffed.

Large quantities of the Spanish green olives have been imported into the United States each year. Based on comparative estimates, the United States has imported approximately twice as many Spanish green olives

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as the quantity of canned ripe olives produced in California, although in recent years the ratio has approached unity as shown in table 1.

The Spanish green-olive industry is confined to a small area in southern Spain, surrounding the city of Seville, where the fermented green olives are produced primarily for export trade. The Sevillano and Manzanillo varieties are the only olives used extensively for green pickling, although many other varieties are grown for other purposes and may be substituted to some extent for the more desirable varieties, during short-crop years.

Harvesting.—The harvesting season usually begins between September 15 and October 1, and lasts until approximately November 1. The maturity of the fruit at time of picking is such that the olive is of full size but not sufficiently ripened to become tender. The color of the fruit at the time of harvesting for green pickling is preferably green to straw yellow, although fruit faintly colored may be used. Riper fruit is undesirable because the flesh softens and the color becomes grayish during the fermentation process.

Handling and Care of the Fruit.—The fruit is picked into a small woven grass or wicker basket suspended on the picker. Extreme care in handling is taken to avoid bruising, for such defects are accentuated during the fermentation process. The fruit is selected for size and maturity as it is picked; this makes several pickings necessary to obtain fruit of uniform quality.

After picking, the olives are transferred to larger baskets, which are carried to sorting tables where ripe or bruised fruit, insect-damaged, or undersized fruit is sorted out by hand. The overripe and undersized fruit is consumed locally and the damaged fruit is pressed for its oil. The grower is required to sort his fruit carefully before delivery to the packer.

The olives are transported from the smaller groves in large, high-wheeled carts, lined with soft grass matting; or in large woven grass baskets in carts, in motor trucks, or on flat cars by train. The grower is generally required to deliver the fruit before it has begun to heat in the baskets after picking. This requirement is intended to minimize infection and subsequent deterioration of the fruit during fermentation.

Preparation for Fermentation.—The fermentation plants are located either in Seville or in the small communities about the city. The plants usually consist of a large walled-in fermentation yard with adjacent buildings housing the lye vats, and facilities for grading, pitting, stuffing, and packing the fermented olives.

On arrival at the factory the fruit is placed in concrete or brick vats lined with cement and treated with lye (sodium hydroxide) solution to

TABLE 1
UNITED STATES IMPORTS OF GREEN FERMENTED OLIVES FOR CONSUMPTION COMPARED WITH PRODUCTION OF CALIFORNIA RIPE OLIVES

Year	Total imports				Imports from Spain			Pack of California ripe olives in cases*	Ratio of total imports to California ripe olives	Ratio of Spanish imports to California ripe olives
	Green in brine	Pitted or stuffed	Total	Cases of 96 buffet/case*	Green in brine	Pitted or stuffed	Total			
	<i>thousands of gallons</i>	<i>thousands of gallons</i>	<i>thousands of gallons</i>	<i>thousands of cases</i>	<i>thousands of gallons</i>	<i>thousands of gallons</i>	<i>thousands of gallons</i>	<i>thousands of cases</i>		
1930.....	5,513	3,307	8,802	1,796				635	2.84	
1931.....	3,325	2,447	5,769	1,177				417	2.82	
1932.....	3,563	2,515	6,078	1,240				379	3.27	
1933.....	2,656	2,284	4,940	1,008				502	2.00	
1934.....	2,659	2,307	4,966	1,014				640	1.59	1.53
1935.....	3,617	2,864	6,481	1,323	2,404	2,296	4,790	553	2.39	2.34
1936.....	3,074	3,609	6,683	1,360	3,489	2,857	6,346	952	1.39	1.89
1937.....	2,676	2,928	5,604	1,145	1,903	3,606	6,509	743	1.54	1.34
1938.....	3,250	2,899	6,149	1,255	1,903	2,922	4,881	603	2.08	1.82
1939.....	3,237	3,153	6,390	1,304	2,432	2,897	5,379	867	1.50	1.33
1940.....	3,902†	3,708	7,610	1,553	3,028†	3,145	6,821	1,135	1.37	1.23

* Data for thousands of cases estimated on the basis of 96 buffet/case equal to 5.8 gallons. A buffet case contains 96 cans of 4½ ounces each, drained weight per can.

† Figures include green and ripe olives in brine. No other data available at present. Data for 1941 not complete.

Sources of data:

U. S. Dept. of Commerce, Foreign commerce and navigation of the United States,
Western Canner and Packer, 34 (5):145-47, 1942.

remove most of the naturally occurring bitterness, which is undesirable and objectionable if very pronounced in the fermented fruit. After lye penetration has gone about two thirds of the way to the pit, the excess lye is removed by leaching the fruit with water.

According to Gracey (1918)⁵ and Cruess (1924) the strength of the lye is adjusted according to the variety and maturity of the olives, the temperature, and other factors; lye concentrations used are 1.6 per cent for the Sevillano variety and about 2.06 per cent for the Manzanillo olives.

The lye solution is added in a quantity sufficient to completely cover the olives in the vats. The olives are prevented from floating by means of heavy grass matting or other floats placed on the surface of the liquid.

Lye penetration is carefully observed by frequently cutting fruit to the pit with a knife. The depth of penetration is indicated by the yellowish-green color of the lye-treated flesh. Alkali indicator solutions such as phenolphthalein are also used. Penetration is allowed to proceed about two thirds of the way to the pit. No set period of time is allowed for lye penetration, since this depends upon the strength of the caustic solution, the temperature, maturity of the fruit, and other factors.

It is recognized, however, that too-deep penetration will destroy all of the bitterness, bleach the color, and may soften the flesh of the olives. Untreated flesh and the bitterness caused by the bitter glucoside *oleuropein* are necessary for a normal fermentation and a finished product of characteristic color, texture, flavor, and aroma. Insufficient penetration results in the production of fruit with objectionable bitterness even after fermentation.

The excess caustic is washed and leached from the olives by several applications of cold water in which the olives are held, with occasional stirring to hasten leaching of the lye from the tissues of the fruit. According to Cruess, the washing period is often less than 24 hours and the olives still contain some lye when they are removed from the vats for fermentation.

Fermentation.—After lye treatment and subsequent leaching the olives are placed in large casks of approximately 170 gallons' capacity called "bocoys." Each cask holds about 1,000 pounds of olives and sufficient salt brine for covering the olives completely. The cask is filled by loosening the upper hoops, removing the head, and adding enough olives to fill to the level of the inner side of the head. Then the head is replaced, the hoops retightened, and the cask rolled into the fermentation yard, where it is usually elevated onto wooden skids to protect it from

⁵ See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

the moist ground. The casks of olives are then filled with a brine containing 10 to 11 per cent table salt (sodium chloride). After a few days the salt content of the brine has decreased to about 5 per cent and the fermentation proceeds rapidly in the warm weather normal for Seville during the pickling season. In unusually warm weather fermentations are completed in as short a time as 1 month; in cool weather 3 months or longer is required.

The normal fermentation is accompanied by a rapid increase in the acidity of the brine caused by the formation of organic acids, chiefly lactic. A large amount of gas is liberated and the casks foam and froth profusely through the side bungs until the fermentation subsides.

During the fermentation the flesh is altered or cured, becomes firm and salty, and light yellowish green in color; at the same time it develops the characteristic flavor and aroma of the Spanish green olive.

Because of the active liberation of gas (chiefly carbon dioxide unless spoilage bacteria are present) and consequent foaming and frothing, as well as the normal expansion and contraction of the brine caused by temperature changes, it is necessary to continually add fresh brine to the casks to prevent undue growth of film-forming yeasts as well as discoloration of the fruit from exposure to the air.

It has been known for many years that the desirable fermentation is the result of the activity of lactic acid bacteria although, to the writers' knowledge, no detailed study of the biology of the fermentation has been reported.

Fermentation must be complete before the olives can be shipped. Alert packers incubate small samples to determine whether gas formation is complete, for, if fermentation is not complete at the time of export, losses will occur when conditions become favorable for continuation of the fermentation.

Preparation for Exportation.—When the olives have been fermented and cured the casks are completely filled with brine, sealed, and stored until needed for shipment.

Before exportation, the olives are carefully graded for quality and size. They are removed from the casks after removal of one head and withdrawal of some of the brine. They are first graded roughly into three sizes by hand-operated screens and then are carefully size-graded by hand and sorted for quality.

The size grades are generally reported as the number of olives per kilogram or per "kilo." The usual size grades for the Sevillano variety are Large, 70–100; Medium, 100–140; and Small, 140–200 olives per kilogram. The range for the Manzanillo variety is 180–400 olives per kilogram.

There are three quality grades: First Quality, Second Quality, and Culls. Not more than 10 per cent of the First Quality olives are allowed to have slight imperfections, while nearly all of the Second Quality olives show some sort of blemish. The Culls are broken, insect-injured, and otherwise seriously imperfect.

After grading, the olives are replaced in the casks used for fermentation and covered with the original brine. The casks are reheaded and any brine lost is replaced with fresh brine containing about 10 per cent salt.

Pitting and Stuffing.—The majority of the stuffed Manzanillo olives consumed in the United States have been pitted and stuffed in the Spanish factories. The graded olives are pitted by hand. Then small strips of canned pimento are folded and stuffed into the pitted olives, also by hand. The stuffed olives are then packed in barrels holding about 48 gallons, covered with fresh brine, and allowed to stand in the sun with the bungs open until fermentation of the pimento flesh is complete. This fermentation takes a month or more and is necessary to prevent fermentation and gas formation during shipment or after the stuffed olives have been packed in glass containers.

After arrival in the United States, the olives are packed in glass bottles and jars. The fancy, glass-packed fruit is prepared by placing the olives individually in the container by means of wooden or metal tongs or with a long, slender, wooden stick. A loose or bulk pack is also made by dropping the olives into the jars at random. After packing by either method, the olives are rinsed with water and drained. This washing is for the purpose of rinsing off small particles of debris adhering to the olives as well as for washing away the microbial residue (cells of yeasts and bacteria) in order to reduce the amount of sediment formed in the bottom of the container or on the olives during storage and to insure a clear brine. The containers are then filled with freshly prepared clear brine and sealed. Formerly corks were commonly used for sealing, but in recent years metallic closures of various kinds have predominated.

Literature pertaining to the fermentation of Spanish green olives is fragmentary. The above discussion has been taken from the writings of Gracey (1918), Cruess (1924), and Bull (1936). The reader is referred to these authors for more detailed information.

Typical scenes in the factory of the Compania Exportadora Española of Seville are shown in figure 1. It is interesting to note in particular the extensive use of hand labor in grading and sorting in the Spanish factory as contrasted with the grading machines and mechanical sorting tables so commonly used in the California olive plants. Figure 3 (p. 16) shows a typical California factory, with fresh fruit being sorted for defects on the sorting tables and graded for size with the mechanical grader.

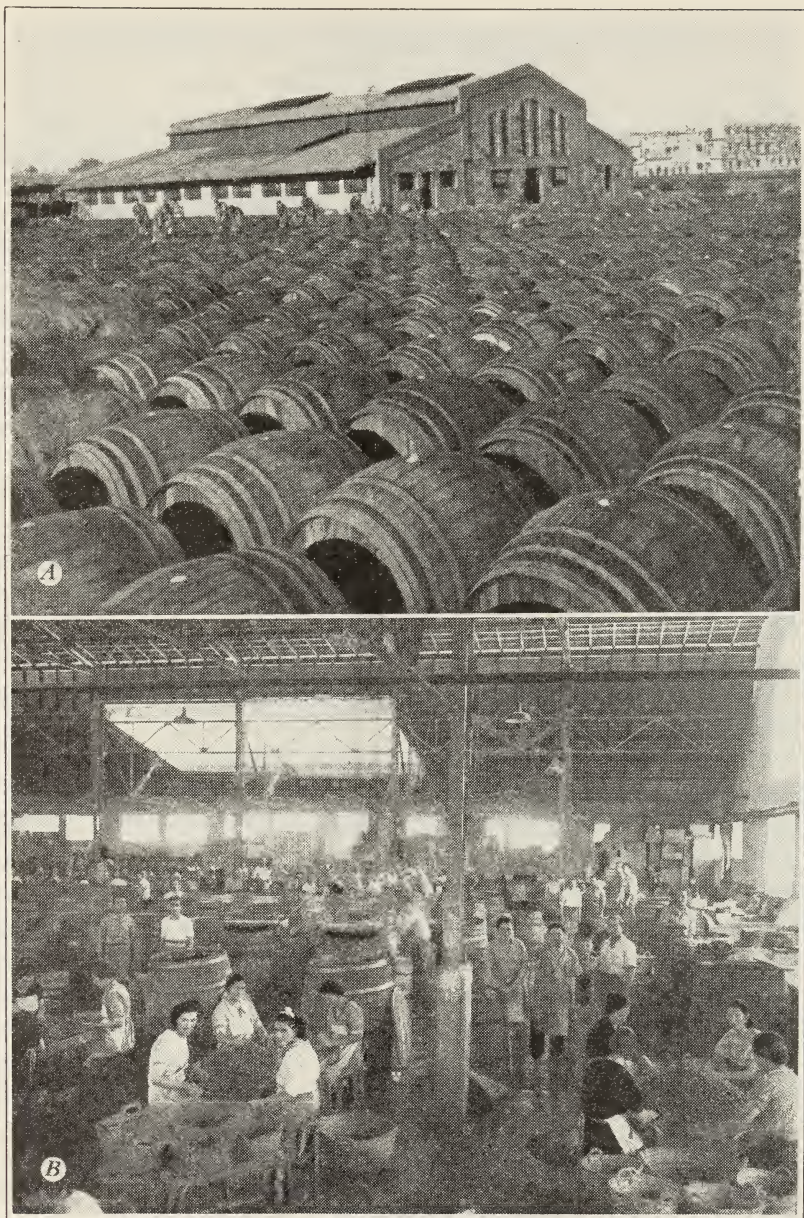


Fig. 1.—Scenes in Seville. *A*, Fermentation yard, with men shown adding fresh brine to the casks. *B*, Grading and sorting the fermented fruit. Note the wicker baskets used for handling the olives. (Courtesy of J. Ungar.)

HISTORY OF THE GREEN-OLIVE INDUSTRY IN CALIFORNIA

The first olive trees in California originated from seed said to have been planted at the Mission San Diego in 1769. The seeds were apparently brought from San Blas, Mexico, by Don Joseph de Galvez, during an expedition to rediscover the port of Monterey. The seedlings thus obtained are the source of the present Mission variety of *Olea europaea* (Lelong, 1890).

The olive was not planted extensively until about 1860, but by 1870 the industry was showing promise of becoming of some importance to California agriculture. Thomas (1859) reported only 503 olive trees in California in 1855. By 1875 the plantings had risen to 11,561 trees old enough to bear fruit. Between this period and 1900 many varieties were introduced and much effort was expended in testing these varieties, principally for oil production. The number of trees of bearing age in 1910 had increased to over 958,000; in 1920, there were over 911,000; by 1930 the number had increased to over 1,600,000; and in 1940 there were at least 1,418,820 trees of bearing age, equivalent to 23,647 acres on the basis of 60 trees per acre. These trees yielded some 60,000 tons of fruit. The important varieties now grown are Mission, Manzanillo, Sevillano, and Ascolano.

Although olive oil was made at the Mission San Diego as early as 1780, the first commercial production of oil in California outside the Missions is said to have occurred at Comulos, Ventura County, in 1871. Pickling, practiced as an art in the missions, on the farms, and in the homes for many years, was of little commercial value until about 1900.

Cooper, Pohndorff, Flamant, Hilgard, and Klee were closely associated with the beginnings of the olive industry in California. Later Paparelli, Hayne, Colby, Shaw, and particularly Bioletti contributed additional knowledge. Early studies were invariably chiefly concerned with the horticultural aspects and with the production of olive oil. Little attention was given to utilization of olives for pickling, and no efforts were made to study pickling extensively prior to 1890.

Directions for pickling ripe and green olives are to be found in the early literature of California agriculture, but the recipes or formulas are sketchy and preservation of olives by pickling was necessarily an art. For some reason the art of pickling ripe olives was studied more extensively than green olives; and with modern technology the canned California ripe olive has gained worldwide prestige. Until recently, however, the pickling of green olives has been of no great importance in California; this is surprising in view of the fact that the Spanish

Queen olive or Sevillano (Gordal) variety was introduced into California some time before 1866 (Dunn, 1866-1867).

The essential basic steps for the art of pickling olives have been known for a long time. Cooper (1882), Flamant (1887), and Pohndorff (1884) wrote directions for pickling green olives before 1890. Paparelli (1890) apparently wrote the first directions published by the California Agricultural Experiment Station.

Paparelli recommended the use of a solution of lye of 6° Baumé (approximately 4 per cent NaOH) for removal of the undesirable "tartness" of the green olive. After contact of several hours, when the fruit was "well cured" by the lye, this solution was drawn off and replaced by soft water, which was to be changed several times during 3 or 4 days until the wash water was completely clear. Then the olives were placed in kegs with a brine consisting of 1 ounce of salt and 13 ounces of water for each pound of olives; or a gallon of brine containing 10 ounces of salt to 10 pounds of olives. In this manner the olives were preserved very well and were "in a condition to be used in from 1 to 2 months, according to taste." Probably very little fermentation occurred in this procedure, since lye treatment and subsequent leaching would remove most of the fermentable sugars.

Some contributions to the art of pickling green olives were made by various investigators, but until the work of Cruess (1930) little knowledge of the fermentation of green olives had appeared in print in the United States or elsewhere.

It is difficult to tell when the first attempts at commercial production of green fermented olives were made in California. Wetmore (1919) apparently was among the first to attempt such production and made his first pack in 1889. It is reported⁶ that about 1900 Mr. Campbell of the American Olive Company, Los Angeles, and Mr. Roeding of the Roeding Fig and Olive Company, Fresno, conducted commercial-scale experiments on production of green fermented olives. At about the same time Mr. A. Adams, Jr., first pickled green olives; later, about 1923, he made Spanish-type green olives at Porterville. Adams has continued production until the present.

The first extensive commercial-scale experiments made to standardize and improve the green fermented olive as produced under California conditions were conducted by Cruess (1924, 1930). These experiments have done much to place the art of pickling green olives on a firm scientific foundation.

Since about 1935 several companies have become interested in the production of this type of olive as a means of saving a part of the crops

⁶ Personal communication from Mr. J. J. Hoey.

not utilized for canned ripe olives or for oil. The increased production shown in table 2 is in large measure the result of conscientious efforts on the part of the whole industry to adapt the fermentation process to conditions prevailing in California. Increased unrest in Europe has also been an incentive although, until recently, the imports of green olives from Spain have been normal.

The prospects during the present war indicate that imports of Spanish green olives may be markedly curtailed. The demand for this product in the United States is well known (table 1) ; and, since it is anticipated

TABLE 2
PRODUCTION OF GREEN OLIVES IN CALIFORNIA

Season	Total green		Spanish type		
	Tons	Gallons*	Tons†	Gallons	Equivalent barrels‡
1935-36.....	2,190	796,363	660	240,000	4,800
1936-37.....	1,200	436,363	500	181,818	3,636
1937-38.....	2,093	761,091	593	215,636	4,312
1938-39.....	5,341	1,942,182	3,841	1,396,727	27,934
1939-40.....	3,000	1,090,909	2,500	909,091	18,182
1940-41.....	6,000	2,181,818	4,500	1,636,363	32,727
1941-42.....	7,000	2,545,454	5,500	2,000,000	40,000

* A gallon is approximately equal to 5.5 pounds of olives.

† Data for 1937 to 1942 estimated, and no segregation into types has been made. For estimation of the Spanish-type pack, the Sicilian pack has been assumed to be constant at 1,500 tons.

‡ A barrel is approximately equal to 50 gallons, or 275 pounds of olives, without the brine.

that the canning of ripe olives will be restricted because of tin-plate shortages, the production of the Spanish-type green fermented olives should be increased if the total olive crop is to be utilized to the best advantage.

PROCESSES USED IN PICKLING SPANISH-TYPE GREEN OLIVES IN CALIFORNIA

The basic steps in production of green olives in this state are essentially the same as those used in Spain, although certain adaptations of the process have been made to meet the prevailing conditions.

Because the production of this type of olives in California has been considered as a means of utilizing surplus fruit not needed for ripe pickling or for oil, and heretofore has been regarded chiefly as a by-product by the olive industry, the progress made in production has been slow and only a few processors have attempted large-scale production. Nevertheless, the preparation of green olives has been the subject of much investigation and it is recognized that they can be made satisfactorily; their quality compares favorably with that of olives

imported from Spain. Production figures for California are shown in table 2.

The chief obstacle to large-scale manufacture of green olives in California has been the high cost of production as compared to the cost in Spain. Too, the canning of ripe olives has required most of the fresh fruit, since canned ripe olives have been the major product of the California industry for many years.

Varieties for Green Pickling.—In previous studies Cruess (1930) has shown that the principal varieties grown in California varied markedly in suitability for green pickling. The Sevillano, Manzanillo, and Mission were found to ferment satisfactorily and to make a good pickled product. While Mission, Manzanillo, and Sevillano olives in the order named constitute the major varieties of the total olive crop in the state, this order is exactly reversed as respects their fermentability.

The Ascolano variety is of little value for green pickling because the color of the lighter fruit becomes almost white after fermentation, and salt shrivel is severe. Since the Ascolano makes good canned ripe olives it would be better to divert this variety entirely to such production. If, however, this variety must be used for green pickling, Cruess recommends that the fruit first be sorted carefully to remove most of the light-colored fruit.

The Barouni variety is of doubtful value. Although the Barouni ferments almost as rapidly as the Sevillano olive, the texture of the finished pickle is often woody and tough, and the fruit from some localities, particularly the Fair Oaks district, has been observed to discolor and turn reddish near the pit in the lye treatment. On the other hand, some excellent green olives have been made from Barouni fruit in the Visalia area. Care and experience is required to properly pickle the Barouni.

Maturity.—In California, the harvesting season extends for a period similar to that prevailing in the Seville district of Spain. It has been customary in this state to receive olives for green pickling throughout most of the harvesting season, which sometimes lasts until well after November 15, unless frosts damage the fruit before the crop intended for pickling (ripe or green) has been harvested. In spite of this custom it has been recognized that the fruit should be green to straw yellow in color at time of picking in order to obtain the best finished product. Most of the olives destined for green pickling are harvested between October 1 and November 15, the time depending upon the locality, the variety of fruit, and other factors.

Olives which are too green (picked about September 15 or before) do not ferment well. Those picked near the end of the harvesting season

often become undesirably softened or discolored on fermentation; they may have internal rot, insect injuries, and other imperfectly known defects which contribute to cullage and spoilage during fermentation. Olives picked late in the season are prone to spoil more easily during the early stages of the pickling process.

Unfortunately, no readily adaptable method for determining maturity has been developed and it is left to the fieldmen to estimate maturity by empirical means.

Harvesting and Transportation.—Fresh olives are extremely sensitive to bruising; and care must be taken in picking the fruit, as well as in subsequent transportation to the factory and handling during preparation for pickling.

To avoid undue injury, buckets made of canvas should be more commonly used in picking. The buckets of fruit are emptied into boxes. These are fitted with cleats to protect the olives against bruising and crushing when the filled boxes are stacked and transported. The fruit lugs contain about 40 to 50 pounds of olives.

In California it is not always feasible to make several pickings from the trees or to select the fruit as carefully for size and maturity as is done in Spain. Often, when the majority of the fruit in an orchard is ready for harvesting, the picking is started and continued until all the fruit destined for ripe or green pickling has been gathered. That part of the crop not included in the first pickings may be later gathered and pressed for oil. The fruit in the tops of the trees generally is picked first, as it is often riper. Harvesting costs are very high as compared to those prevailing in Spain; pickers may be scarce, and the grower cannot afford the costs necessary to employ the amount of hand labor customarily used in that country. Furthermore, since the canned, ripe, pickled olive is the chief product of the olive industry it has been customary to pick the fruit when it is most desirable for ripe pickling; olives destined for green pickling are sorted out at the factories unless the company concerned packs an appreciable quantity of green pickled olives.

The picking practices are influenced greatly by the farm price of the fruit and the availability of harvesting labor. All recognize the value of careful harvesting and selection of the fruit for maturity, but under conditions which prevail during some seasons it is impossible to maintain the desired standards for picking. Sevillano olives receive more care in harvesting than the Manzanillo, and several pickings are customarily made. Other varieties may or may not receive the same attention.

The harvested fruit is transported to the pickling plants by motor

vehicles. Light or heavy trucks are used, according to the quantity of fruit and the distance it is to be hauled. Some olives are carried as far as 350 miles (from the Corning area to Visalia). Use of the cleated lug boxes shown in figures 2 and 5, *A*, allows the fruit to be transported without undue bruising and crushing. Generally, however, green olives are pickled within a few miles of the groves, as it is recognized that long transportation is undesirable.



Fig. 2.—Harvesting olives in California. Note the cleats on the lug boxes designed to prevent bruising of the olives when the boxes are stacked.

Sorting and Grading.—In most factories the olives are graded for size, color, and defects. Sorting for defects and size-grading are generally the first operations. Grading machines consisting of moving, diverging steel cables are most commonly used. The cable grader is popular because it is accurate and of large capacity (fig. 3). A roller grader of new design is also coming into use. The olives are graded for size before pickling in order that the action of the lye may be uniform, particularly in the ripe process. Some of the plants receiving green fruit in abundance at the height of the season forego size-grading of the fruit until after the pickling process is completed. This is done to reduce costs by limiting the number of gradings; it also speeds up handling of the fresh fruit and reduces bruising. In most cases, however, the fruit is sorted and graded both before and after fermentation.

It is customary to sort the olives carefully for color into three grades: black, cherry-red, and green fruit. The green fruit is then used for green or ripe pickling according to demand, and the black and cherry-red fruit is used for ripe pickling or by-products.

Whereas size-grading is entirely mechanical, grading and sorting for color defects has to be done by hand. Sorting tables with endless belts are used, and women sort the fruit as it passes by on the belts. Although still an expensive operation, sorting by this means is a decided improvement over the hand labor used in Spain.

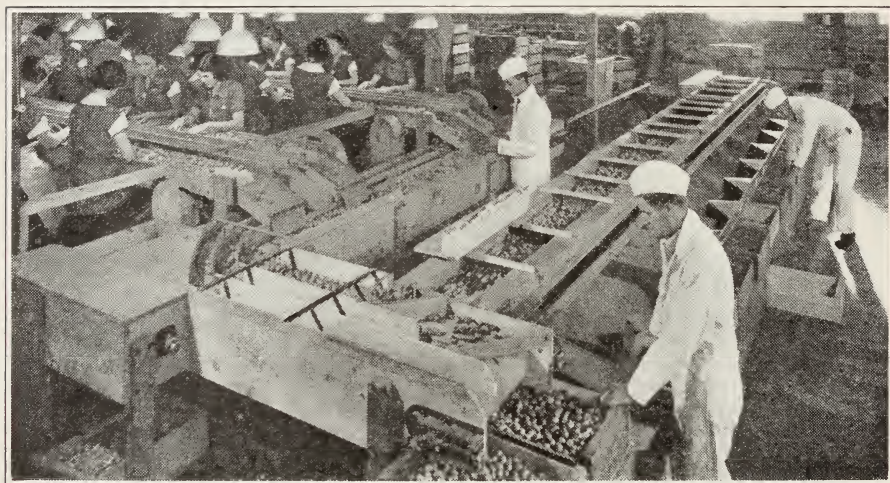


Fig. 3.—Sorting and grading fresh olives.

At present, the size grades established for California canned ripe olives are used as sizes for green olives (fig. 4). Originally the unit of measure for designation of the different size grades was $\frac{1}{16}$ inch. The largest size was at least $1\frac{7}{16}$ inches in diameter, and the smallest commonly used for pickling was $1\frac{9}{16}$ inch in diameter. Since, however, the different varieties vary significantly in shape, standard designation of size is now based on count per pound as shown in table 3. Green olives smaller than any designated in figure 4 are often pickled for use in cocktails and other mixed drinks. It so happens, however, that the mechanical graders sort the fruit into weight grades which approximate the original size grades. Sizes for Spanish fruit are also shown in the table.

Lye Treatment.—Fresh, unpickled green olives are intensely bitter and, unless treated with lye to remove this condition, are very objectionable to the taste. The bitterness was thought originally to be due to the

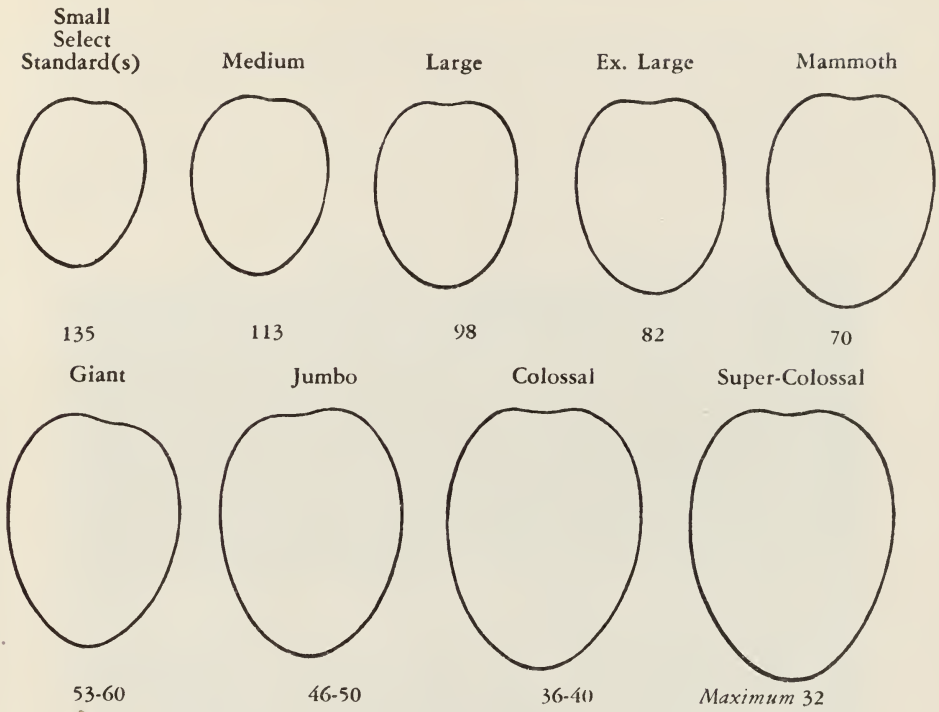


Fig. 4.—Size grades for olives. The numbers indicate the average number of olives per pound. See discussion in the text.

TABLE 3
SIZE GRADES OF CALIFORNIA OLIVES BASED ON COUNT PER POUND
TOGETHER WITH SPANISH SIZE GRADES

California standard size grades	California count per pound	Spanish count per kilo*	Equivalent Spanish count per pound
Super-Colossal.....	32†	70- 80	31- 36
Colossal.....	36-40	80- 90	36- 41
Jumbo.....	46-50	100-110	45- 50
Giant.....	53-60	120-130	55- 59
Mammoth.....	70	150-160	68- 73
Extra Large.....	82	170-190	77- 86
Large.....	98	200-220	91-100
Medium.....	113	240-260	109-118
Small, Select, or Standard(s).....	135	280-300	127-136

* A kilo (kilogram) is equivalent to 2.2 pounds.
† Maximum number allowable.
Sources of data:
Correspondence with California Olive Association.
California Ripe Olive Standardization Act as amended 1933 and 1935.
(Section 870 of Agricultural Code, as amended.)

presence of gums or tannin materials in the flesh of the olive. It is known now to be due to a glucoside, oleuropein, named by Bourquelot and Vintalesco (1908), and isolated and studied in purified form by Cruess and Alsberg (1934).

The glucoside may be removed by leaching the olives with water for a long period of time or destroyed rapidly by hydrolysis with dilute alkali solutions; the latter method is used in California.



Fig. 5.—Showing various stages in the green-pickling process. *A*, Filling a tank with olives. *B*, Full tank ready for lye treatment. *C*, *D*, Filling barrels with olives after lye treatment and washing. (*C*, By courtesy of Pacific Olive Company.)

For lye treatment, the fruit is placed in shallow vats or tanks and covered with a dilute solution of commercial-grade sodium hydroxide. Lye-treated green olives darken rapidly on exposure to the air. To prevent undesirable oxidation and darkening, the olives are kept submerged by use of a slat grating, which fits into the tank or vat and floats on the surface of the lye solution. Some processors use their ripe-pickling vats (cement or wood) for treating the olives with lye. Larger producers of olives customarily use large redwood tanks for the treatment and subsequent washing (fig. 5).

Heating occurs when lye is dissolved in water. Provision should be made so that the solution may be chilled to 60°–70° F before coming in contact with the olives; this is desirable to control the rate and depth of penetration more easily.

Lye solutions for treating green olives vary between 1.25 and 2.00 per cent, as used commercially in California; no single concentration is in use or advocated. Solutions of 2.00 per cent must be used with care, for they often cause blistering and softening, particularly when used to treat the Sevillano variety. All large varieties must be lye-treated with care.

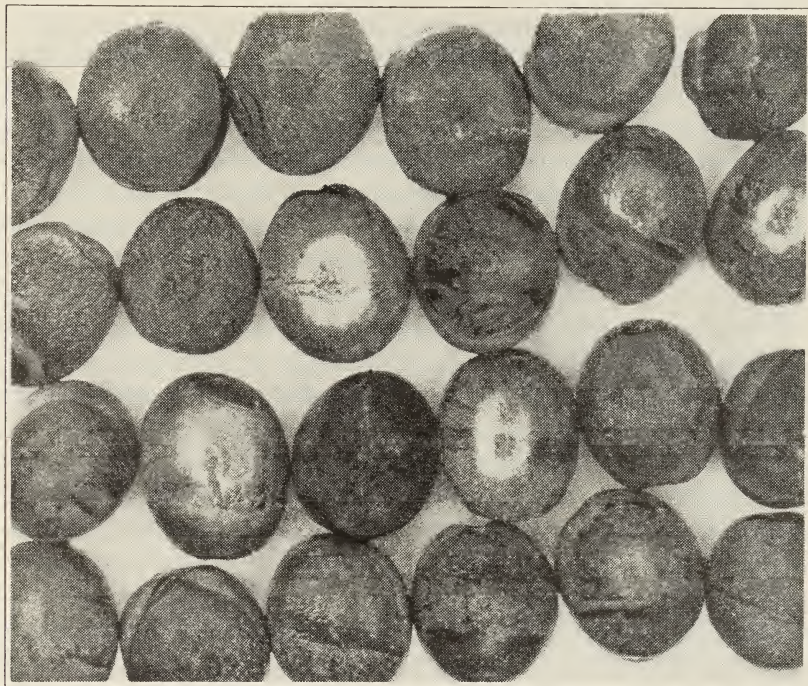


Fig. 6.—Showing variation in depth of lye penetration under commercial conditions. (Courtesy of H. G. Schutt.)

The solution is allowed to penetrate about one half to three fourths of the way to the pits of all the fruit treated. If the solution is too strong, or its application too prolonged, all of the bitterness is destroyed and the flavor, texture, and color of the finished olives are likely to be inferior. A small amount of untreated flesh should remain, for the small quantity of bitterness left is essential to give the fermented fruit part of its characteristic flavor.

The depth of penetration of the solution may be followed by observing the depth of the yellowish-green color of the lye-treated flesh. Commonly, however, depth of penetration is tested by applying phenolphthalein indicator solution to the cut surface of olives (fig. 6). The indicator solution (1 per cent phenolphthalein in neutralized, denatured

alcohol) is equally useful in observing lye penetration and progress of washing to remove the excess lye after treatment. A few drops placed on the cut surface turns from colorless to pink or red, according to the amount of lye present.

The time required for lye penetration cannot be standardized. The time varies from about 4 to 12 hours or even longer under certain conditions. Penetration depends upon too many factors not easily controlled, such as concentration of lye, temperature of the solution, size, variety, and stage of maturity of the fruit. This has been shown adequately by Cruess (1930) and is common knowledge in the industry.

Washing to Remove the Excess Lye.—It is necessary to remove the excess of lye by washing and leaching with water before the lye-treated fruit is barreled and brined for fermentation. The removal of caustic is accomplished by replacing the lye solution on the olives with water, which is changed at intervals over a 24- to 48-hour period of time. There is no uniformity in the industry regarding water-changing intervals; they vary between 3 and 6 hours during the day and may reach 10 hours during the night.

Several factors influence the time required to wash the excess lye from the fruit: the concentration of the lye in the original solution used for lye treatment; intervals between changes of wash water; size and maturity of the fruit; and the chemical composition of the wash water. The tendency at present is to shorten the washing period in order to minimize graying of the color.

Generally the olives contain some lye when they are removed from the vats for barreling. Cruess (1924) observed that in the Spanish process a strong reaction for free lye was obtained when the washed olives were cut and treated with phenolphthalein solution. Cruess, however, advised washing until all or nearly all of the treated flesh was free from lye. On the basis of later experiments made in California, Cruess (1930) found that it was not necessary to remove the last traces of lye from all of the olives, for when 75 to 80 per cent of the fruit gave no reaction with phenolphthalein and the remainder gave a faint red color, the lot fermented satisfactorily. In experiments conducted since 1937 by the present authors, washing has proceeded to various pH levels between about pH 7.0 and pH 8.5 without noticeable effect on the fermentation. Under conditions prevailing in certain plants in California washing to remove most of the lye is impossible, for the water available there is in itself alkaline enough to give a strong reaction with phenolphthalein. (Phenolphthalein indicator solution changes from colorless to pink or red at pH 8.2 to 8.4.)

It is absolutely necessary to avoid exposure of the lye-treated olives to

air for any length of time beyond that necessary to remove the lye solution and replace it with wash water, or the wash water with brine. Lye-treated olives darken rapidly on exposure to air, and the resulting gray to brown color does not completely disappear during fermentation. Likewise, the washing period must not be unduly prolonged or the skins of the olives will become grayish in color and result in a product of poor commercial grade, or unsalable.

Violent aeration must be avoided in filling the tanks or vats with fresh wash water, for the dissolved, occluded, and entrapped air may darken the olives severely. Exposure to the air between changes of wash water during filling of the barrels must also be brief as possible. These conditions must be universally adopted during the washing process if color of the lye-treated fruit is to be preserved at its best.

Barreling.—Immediately after washing, the olives are placed in barrels, covered with salt brine, and rolled into the fermentation yard where they undergo lactic acid fermentation.

Unlike the Spaniards, who use large casks of about 170 gallons' capacity, the Californians customarily use barrels containing about 50 gallons of fruit in brine (about 275 pounds of olives and 20 gallons of salt brine).

To fill the barrels, the upper hoops are loosened, one head removed, and the washed olives added to the level of the inner surface of the head. The head is replaced, the hoops tightened by hand or by mechanical hoop tighteners (fig. 7), and brine poured in immediately.

Oak barrels have been used by preference as they are sturdier, not so apt to leak as spruce barrels, and withstand handling and shipment well. Paraffin-lined spruce barrels are not satisfactory for pickling green olives; they generally impart an undesirable flavor and aroma to the product and lack the sturdiness of those made of oak.

Some new oak barrels have been used but for several years it has been customary for those packers pickling green olives to use rebuilt oak barrels previously used for aging and shipping distilled spirits. These barrels have been completely cleaned and recoopered and are entirely satisfactory, as indicated by their extensive use.

Second-hand oak barrels from other sources have been used by some picklers; but unless such barrels are cleaned thoroughly and carefully recoopered, in the end they often cost as much as or more than rebuilt or new barrels. Used barrels must be thoroughly cleaned with sodium carbonate or other washing compounds, either by soaking out with dilute solutions of the washing compound and rinsing with water or, preferably, by applying hot water containing a washing compound. This cleansing treatment must be followed by thorough leaching with water,



Fig. 7.—Brining and heading of olive barrels. *A*, Driving hoops by hand after inserting the barrel head. *B*, Caulking a leaking barrel with dried tule leaves. Note the workman with brining hose. *C*, Mechanical hoop tightener. *D*, Rebrining barrels in fermentation yard.

and finally by treatment with clean, live steam. *The barrels must be treated until all off-odors have disappeared.*

War priorities have restricted the use of metals and it is to be expected that companies formerly using only barrels bound with galvanized hoops will be fortunate to obtain barrels bound with unpainted iron hoops. It is recommended that all such untreated hoops be covered with a good-quality paint before the barrels are used. Sometimes fermented green olives are stored in the same barrel for considerably over a year; the salt brines used for pickling are very corrosive and many times the unpainted, ungalvanized hoops corrode through and have to be replaced. Time and materials can be saved by painting all raw iron hoops before the barrels are filled with olives and brine.

Brines and Brining.—The Sevillano is most commonly used in the Corning area, being the main variety grown there, whereas in the Lindsay-Visalia-Porterville area the Manzanillo is more common and, consequently, used more extensively for green pickling; the Mission variety has been used in some quantity during the past two seasons.

From the practical viewpoint, it has been known for a long time that salt may be used as a preservative, if present in sufficient concentrations; but only rather recently has it been learned, from the scientific standpoint, that different microorganisms tolerate different concentrations of salt.

Olive picklers are cognizant of the fact that if the salt brines used with their olives are maintained at a level of roughly 28° salometer (about 7 per cent salt), microbial spoilage is reduced to a minimum without eliminating the desirable lactic acid bacteria. The Manzanillo and Sevillano olives, however, react differently to salt brines; and separate processes have been developed for their brining and fermentation.

The lye-treated Manzanillo olive does not shrivel badly when immersed in brines containing rather high concentrations of sodium chloride up to about 15 per cent salt (about 60° salometer). The Sevillano fruit, on the other hand, is subject to salt shrivel; and processors generally start this variety in a brine containing a low concentration (ranging from about 5 to 6.25 per cent, or 20° to 25° salometer) and then increase the salt content of the brine slowly over an indefinite interval of several weeks to several months.

Manzanillo olives are commonly started in brines containing salt in concentration of from 10 to 15 per cent (40° to 60° salometer). At the end of the period of stabilization the salt content of the brine, through the process of diffusion, will have decreased to an extent dependent upon the original concentration of salt in the brine and other factors. The final concentration of salt in the brine will range between about 6 and

9 per cent salt; this is then adjusted to and maintained at 7 to 8 per cent throughout the course of the fermentation.

Sevillano olives, as previously stated, are commonly started in brines containing much less salt—5 to 6.25 per cent (about 20° to 25° salometer) or even less. As the salt content of the original brine decreases during the period of stabilization, the final level of salt in the brine will range between about 2.5 and 4 per cent (about 10° to 16° salometer).

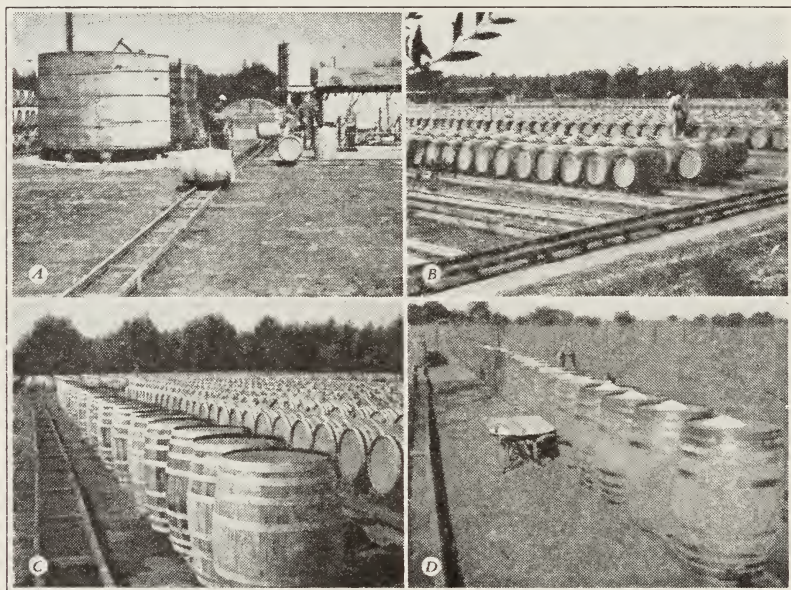


Fig. 8.—Showing various stages in the green-pickling process. *A*, Rolling a filled and brined barrel into place in the fermentation yard. *B*, Barrels of olives undergoing fermentation. *C*, Method of increasing salt content of brines for Sevillano olives by letting brine overflow the barrel heads. This practice is used in the Visalia-Porterville-Lindsay area. *D*, Addition of salt to the brine in the flooded barrel heads. (*D*, By courtesy of W. V. Cruess.)

The salt content then is increased gradually to between 6 and 8 per cent salt and maintained in that range throughout the remainder of the fermentation.

The salt is added in small quantities at indefinite intervals of time. It may be added directly to the brine through the side bung of the barrel resting on one side in the fermentation yard, or placed in the brine covering the head of the upright barrel of olives. In the latter case the head is prepared with two small holes through which the slowly dissolving salt diffuses into the surrounding brine (fig. 8). After the brine has been increased to the desired concentration, these small top openings are plugged securely and the barrel placed on its side; thereafter the

brine is added through the side bung as needed to replace the brine that has been lost through various causes.

Some have attempted to fit the oak barrels with 6-inch head bungs for use in pickling the Sevillano olives, believing that the large bung would eliminate cooperage and facilitate brining. The barrels thus made are not sturdy; leakage is troublesome; and if the barrels are placed on end to prevent leakage, the bungs soon become loosened and permit rapid evaporation with formation of a head space, resulting in deterioration of the olives from exposure to the air and growth of yeasts and molds.

Once the fermentation has started, the bacteria and yeasts cause the

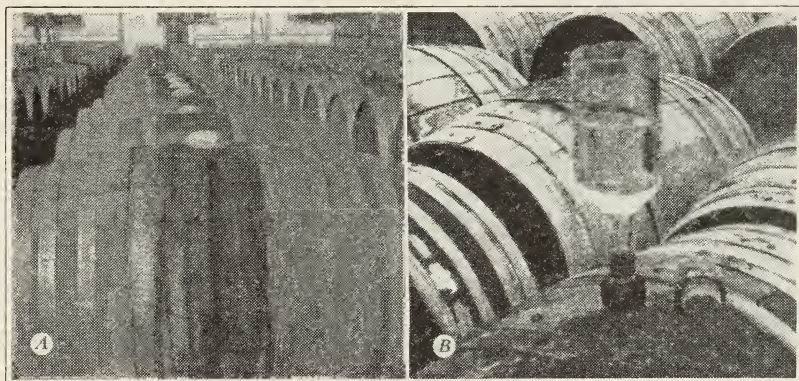


Fig. 9.—*A*, Barrels of Sevillano olives using side bungs for addition of salt, as practiced in the Corning area. Fermentation is in process as indicated by the overflowing froth and brine. *B*, An experimental fermentation bung. Note the cellar bung alongside; this is discussed in the text.

production of large quantities of gas, principally carbon dioxide unless spoilage-producing bacteria are present to cause the evolution of hydrogen. During the stage of violent evolution of gas a considerable quantity of brine is lost by frothing (fig. 9, *A*). Temperature fluctuations causing expansion of the brine account for loss of additional brine.

Two of the commonest causes of inferior-quality green fermented olives are loss of color and "yeast spots"; both are, at least in part, the result of failure to keep the fermenting fruit covered with brine and the barrel completely full at all times.

To preserve the color of the layer of olives exposed to the air through loss of brine it is essential to rebrine the barrels frequently—at daily intervals if necessary. Furthermore, if the barrels are not kept full of brine at all times yeasts are encouraged to develop in tremendous numbers. The activity of these yeasts dissipates the lactic acid formed in the fermentation, thus making the olives more susceptible to spoilage. Their abundance may also result in colonies of yeasts under the surface of the

skin of the fruit, causing the defect known in the industry as "yeast spots."

Several attempts have been made to devise fermentation bungs which will keep the barrels full of brine at all times, allowing for expansion due to temperature fluctuations and preventing carryover of brine that results from violent evolution of fermentation gases. The principal idea prevailing has been to fit the side bung of the barrel with a closure having a small hole through the center of which may be inserted a funnel-type container holding one to several quarts of brine. These "fermentation bungs" are shown in figure 9, *B*. Others have had good success with long oak "cellar bungs" commonly used in the wine industry.

The Fermentation.—The barreled, brined olives are rolled into place on skids in the fermentation yard where they undergo a lactic acid fermentation. The major portion of the fermentation takes place in the presence of from 4 to 6, or even 8, per cent salt and requires from 6 to 10 or more months for completion unless the fermentation is controlled by acceleration of acid production by incubation and other means.

It has been generally believed that lactic acid bacteria are responsible for the fermentation of Spanish-type green olives, but no detailed studies of the microbiological and chemical changes are recorded in the literature.

It has been generally recognized for some time that the Manzanillo olive does not ferment as readily as the Sevillano variety; this variation is partly the result of differences in chemical composition of the two varieties and partly the result of methods of preparing the fruit.

To promote fermentation simple hexose sugar or other fermentable material must be present for the bacteria to convert to lactic acid. That there are marked differences in sugar content of several varieties of olives has been shown by Nichols (1930), who found that the Sevillano, Ascolano, and Barouni olives were high in sugar; the Mission and Manzanillo varieties contained an intermediate amount. Data taken from Nichols' work are shown in table 4.

Cruess (1930) has shown that lye treatment and washing have marked effects on the amount of fermentable material left in the olives; he found that the loss in total reducing sugars plus mannitol (all fermentable material) during lye treatment and subsequent washing was about 65 per cent of that originally present. Furthermore, the natural acidity of the olive flesh was found to be almost completely lost by the lye treatment, and so practically all of the acidity in the fermented fruit had to result from conversion of the sugars and mannitol left in the fruit at the time of brining.

Picklers customarily treat the Manzanillo variety more severely with lye than the Sevillano; this is because the former contains more bitterness than the latter. Cruess and Alsberg (1934), in connection with a study of the chemical properties of oleuropein, found that the Mission variety was highest in content of bitterness and the Sevillano variety lowest. They also found that the Manzanillo and Barouni fruits were comparatively rich in content of the bitter principle, whereas the Ascolano was poor. They observed, furthermore, that the green fruit contained more bitter glucoside than tree-ripe olives regardless of variety.

TABLE 4
PERCENTAGE OF TOTAL SUGARS IN OLIVES OF DIFFERENT VARIETIES*

Variety	Average per cent sugar on wet basis			Average per cent sugar on dry basis		
	Entire season	Early season	Late season	Entire season	Early season	Late season
Ascolano.....	5.54	5.48	5.66	18.2	19.0	16.5
Sevillano.....	4.09	4.87	2.92	16.8	21.1	10.4
Barouni.....	4.59	3.56	4.84	13.3	15.2	12.8
Manzanillo.....	2.96	2.48	3.45	9.8	9.4	10.3
Mission.....	3.68	3.62	3.74	9.5	10.6	8.4

* Data from Nichols (1930).

Thus it is readily seen that the chemical composition of the fresh green olive, in part at least, determines its fermentability; the Manzanillo variety, poor in sugar and rich in bitterness as compared with the Sevillano fruit, must receive more drastic lye treatment and consequently lose more fermentable substances.

The remaining reason for the difficulty with which the Manzanillo fruit ferments is to be found in the high concentration of salt solution commonly used in commercial practice, for this variety, and the effect of the salt on the growth and activity of the lactic acid bacteria.

Sometimes it is necessary to add fermentable material in order to obtain a satisfactory fermentation. Sugar in the form of glucose (commercial Cerelese or corn sugar) is commonly used. The Manzanillo olives are commonly brined with 40° to 60° Salometer brine, whereas the Sevillano olives are placed in 15° to 25° Salometer brine. If the olives contain enough fermentable material after lye treatment to cause the pH of the brine to drop to pH 4.0 or below, addition of supplementary sugar is not necessary. Many picklers of Sevillano olives do not add sugar at all, but those who process Manzanillo fruit generally find it necessary to add sugar and to control the fermentation in other ways. (For a detailed discussion see the section "Control of Fermentation.")

INVESTIGATIONS ON THE BACTERIOLOGICAL AND CHEMICAL CHANGES OCCURRING DURING FERMENTATION

The recent increase in the pickling of green olives in California has been accompanied by many microbiological problems. To facilitate production as well as to control the fermentation to eliminate microbial spoilage it was necessary to investigate the microbiological and chemical changes occurring in the green-olive fermentations.

Study in this field was initiated at the beginning of the 1937-38 olive season. The first investigations were exploratory in nature, for it was necessary to find suitable media and develop certain manipulatory techniques which would give an accurate picture of the microbial populations growing in the various fermentations. During subsequent pickling seasons, various lots of olives were fermented in the laboratory and the brines examined for changes in types of microorganisms as well as the accompanying chemical changes.

Experimental Methods.—The laboratory experiments were conducted in 5-gallon oak kegs and in glass containers of 2-quart or 1-gallon capacity—all fitted with tight closures. A large number of tests have shown that small samples of olives (2 quarts up to 2 gallons) can be fermented so that the chemical and bacteriological changes parallel those occurring in the 50-gallon barrels of olives pickled on a commercial scale. This is possible when care is taken to keep the small fermentations closed to prevent undue growth of yeasts and contamination with other microorganisms, yet the escape of gases is permitted. Smaller lots do not always give satisfactory results.

Samples of brine were taken with sterile pipettes from the various lots of fermenting olives, which previously had been thoroughly agitated by shaking and rolling to insure uniform distribution of microorganisms and chemical constituents in any aliquot of the brines.

During the early stages of the investigation the methods used for estimation of the microbial populations were essentially the same as those used earlier by Pederson (1929, 1930, 1936, 1938) and others who have investigated the fermentation of sauerkraut and cucumbers.

Experience with the lactic acid bacteria from fermenting olives and spoiled wines soon showed, however, that yeast-infusion agar containing 2 per cent glucose was the most satisfactory medium for routine estimation of the numbers of lactic acid bacteria, particularly when the medium was adjusted from pH 5.0 to 5.5 before sterilization and prepared to contain 2 per cent agar. This medium was satisfactory for the

growth of the lactic acid bacteria and yeasts commonly found in the brines from fermenting olives.

The Gram-negative bacteria (spoilage types) were estimated by using Levine's eosin methylene blue agar. The Gram-negative counts included many bacteria other than the common coliform organisms (genera *Escherichia* and *Aerobacter*) responsible for decomposition of olives. None of the common differential agar media used were completely satisfactory for estimation of the numbers of Gram-negative bacteria. Yeasts and molds often grew on the agars and it was then impossible to get very accurate counts. Attempts to use various modifications of the Breed counting technique were entirely unsatisfactory. However, as the coliform bacteria are generally prevalent in the brines during the initial stages of the fermentation their estimation was of paramount importance; Levine's eosin methylene blue agar incubated at 37° C for 24 to 36 hours gave an accurate picture of the fate of these spoilage bacteria.

Plate counts were made at time of brining the olives and at various intervals thereafter until the experiments were terminated.

The agar media for estimation of numbers were contained in 20- to 25-milliliter (cubic centimeter) portions in 6-ounce, screw-capped, uncalibrated, glass prescription bottles. It was more convenient and economical to use these bottles than petri dishes. The counts thus made were found to be as accurate as those made using petri dishes.

Following the usual aseptic methods, appropriate dilutions of the brines were inoculated into the two media contained in the bottles. After agitation, to insure uniform distribution of the microorganisms, the tightly capped bottles were placed on their sides until the agar had hardened; and then they were stored in the incubator until the colonies had developed sufficiently to be counted. The yeast-infusion agar was incubated at 30° C for 4 to 5 days, whereas the eosin methylene blue agar was incubated at 37° C for not more than 36 hours.

After the proper interval of incubation the colonies on the agar in the bottles showing colonies were counted and the populations of microorganisms estimated as numbers of Gram-positive or Gram-negative bacteria per milliliter. Yeasts were detected by examination of colony peripheries under the low power of the microscope.

To investigate changes in types of the lactic acid bacteria and the undesirable spoilage bacteria occurring in the fermenting brines, colonies were picked from the agar media immediately after the counts had been made. An attempt was made to collect all different colony types growing on each medium. The colonies were picked and transferred into sterile liquid media. All colonies picked from the yeast-

infusion agar were transferred to sterile yeast-infusion broth containing 2 per cent glucose. All colonies picked from the eosin methylene blue agar were transferred to nutrient broth. All isolated colonies were subsequently purified by the plating technique. Petri dishes were used for purification, because the bottles were not satisfactory for such procedure. The Gram-positive organisms were purified on yeast-infusion dextrose agar. The Gram-negative organisms were purified on eosin methylene blue agar. The purified Gram-positive isolates (*Leuconostoc* and *Lactobacillus* types) were carried in stock culture in liver-infusion broth containing liver particles, whereas the Gram-negative cultures were carried in nutrient agar slants.

The lactic acid bacteria were identified by the use of the methods suggested by Orla-Jensen (1919), Hucker and Pederson (1930), and Pederson (1929, 1930, 1936, 1938). The methods suggested by Levine and his students (1921, 1933, 1934) were used for classification of the coliform bacteria. The remaining Gram-negative bacteria were classified as accurately as possible according to the manual by Bergey (1939).

The chemical examination of the brines consisted of determination of total acidity by titration; determination of sodium chloride content by titration; and determination of pH by the use of the quinhydrone or glass electrode. In the experiments between 1937 and 1940 the quinhydrone electrode was used routinely for pH determinations; since 1940, the glass electrode has been used.

Total acid content of the brines was determined by titration of an aliquot portion (hot) of brine with 0.1N sodium hydroxide and expression of the acidity in terms of grams of lactic acid. Total sodium chloride content was determined by titration of an aliquot of brine with silver nitrate according to the methods of the Association of Official Agricultural Chemists (1936).

The Bacteria Found in Normal Fermentations.—In normal fermentations of olives the floral sequence may be divided into three stages: primary, intermediate, and final; the bacterial and chemical changes occurring in fermenting olives will be considered in that sequence.

The Primary Stage of Fermentation.—During the early part of the primary or initial phase of the fermentation of green olives a great many unrelated microorganisms were isolated. These included yeasts, molds, and bacteria which are found widely distributed in nature in water, soil, and the air. At the onset of this first stage of fermentation the desirable lactic acid bacteria are far outnumbered by the microorganisms which play no readily obvious role in the fermentation, but which may contribute to deterioration of the olives if the fermentation does not proceed in a normal manner. The majority of the organisms

present during the initial phases of the fermentation are Gram-negative bacteria of the genera *Aerobacter* and *Pseudomonas*. Yeasts are present also, but only small numbers of lactic acid bacteria, for the most part low-acid-tolerant representatives of the genera *Streptococcus* and *Leuconostoc*. A few cultures of Gram-positive micrococci were isolated. Very few isolates of *Lactobacillus* have been recovered during the initial phase of the fermentation.

The primary stage of fermentation is the most important phase of the green-olive pickling process. If the fermentation proceeds in a normal fashion the spoilage bacteria present will soon be eliminated as the result of acid production by the lactic acid bacteria. If for some reason, however, the fermentation does not start normally, the undesirable organisms of the following groups may predominate and spoil the olives: the coli-aerogenes group (principally *Aerobacter* types); butyric anaerobes (*Clostridium butyricum* and others); facultative aerobic, sporeforming bacteria of the genus *Acrobacillus*; or yeasts.

As indicated in table 5, the primary stage normally persists for 7 to 14 days. During this time the salt brine has reached the stabilization point or nearly so; the fermentable materials have become available for the bacteria in the brine; the lactic acid bacteria have begun to develop rapidly; the Gram-negative bacteria have begun to decrease or even disappeared; coincidently, there is a definite, steady increase in total acidity and a corresponding drop in pH.

In cases where the fermentation does not proceed normally, particularly in the case of Manzanillo or Mission olives, the primary phase of fermentation is never completed unless the fermentation is controlled in some manner. Such "stuck" fermentations have been observed to persist in the primary stage for 6 months or more until measures were taken to initiate the lactic acid fermentation or the olives had completely deteriorated.

The Intermediate Stage of Fermentation.—The predominating microorganisms in the intermediate phases of the fermentation are lactic acid bacteria. They include a mixture of the low-acid-tolerant species of *Leuconostoc* and the high-acid-tolerant species of *Lactobacillus*; the *Leuconostoc* types predominate.

The Gram-negative bacteria, already on the decline or completely eliminated during the latter part of the primary stage, have completely disappeared by the end of 12 to 14 days, if the fermentation is normal.

The yeasts have decreased in numbers and the pigmented types commonly found in the primary stage of the fermentation have completely disappeared if the fermentation is kept closed.

The pH of the brine has decreased further; the total acidity has in-

creased and the salt content of the brine has decreased to a constant level indicating complete stabilization.

The intermediate stage of the fermentation lasts for about 2 to 3 weeks. During this period the *Leuconostoc* types have increased, predominated over the other lactic acid bacteria, and finally started to decrease rapidly in numbers, being replaced by the acid-tolerant species of *Lactobacillus*.

The Final Stage of Fermentation.—By about 21 to 28 days or even less time after fermentation of the olives has been initiated, the pH of the fermenting brine has decreased to pH 4.5 or below, the total acid content has increased to 0.25 or 0.30 grams per 100 cubic centimeters expressed as lactic acid; the high-acid-tolerant lactobacilli have also rapidly increased in numbers and replaced the species of *Leuconostoc* which predominated during the intermediate stage of fermentation.

Small numbers of yeasts may persist throughout a properly controlled fermentation, but gas-forming and non-gas-forming species of lactic acid bacteria are the only bacteria present in significant numbers during the final phases. The lactobacilli remain viable and active as long as fermentable material is available or until the acidity of the brine has reached an inhibitory level (above 1.0 per cent total acidity expressed as lactic acid).

At the end of the fermentation the titratable acidity will have reached a level of 0.6 to 1.0 per cent acidity expressed as lactic acid, or even higher, and the pH will have decreased to pH 4.0 to 3.8 or lower if sufficient fermentable material has been available for conversion to lactic and acetic acids.

The Common Species of Lactic Acid Bacteria Found in the Fermentations.—The important floral changes occurring in the brines of fermenting olives have been found to be similar to those reported for the fermentation of sauerkraut by Pederson (1930).

The important species of lactic acid bacteria found to predominate in the Sevillano fermentations were:

Leuconostoc mesenteroides, a gas-forming coccus, which dominates the latter phase of the primary fermentation as well as the early phases of the intermediate stage of fermentation.

Lactobacillus plantarum, a non-gas-forming rod which dominates the latter phases of the intermediate stage as well as the final stage of fermentation.

Lactobacillus brevis, a gas-forming rod found in the latter phases of the intermediate stage as well as in the final stage of fermentation. This heterofermentative species is the most abundant of the gas-forming types.

Lactobacillus buchneri and *L. fermenti*, two gas-forming rods which were encountered infrequently.

As intimated above, the salt concentration of the brines had a marked effect on the total numbers and types of bacteria found in the brines. Thus the fermentations of the Manzanillo variety, which from the start are customarily conducted in the presence of twice as much salt as the Sevillano fruit, have a different bacterial content and different types of bacteria from that of the Sevillano fermentation.

The heterofermentative types of *Lactobacillus* (the gas-forming *L. brevis*, and others) were never recovered from the brines of the Manzanillo fermentations. Their absence may be explained by the work of Orla-Jensen (1919), who reported that gas-forming species of *Lactobacillus* were less resistant to salt than were the species of *Leuconostoc* or the non-gas-forming species of *Lactobacillus*. The observations upon the lactic acid bacteria from green-olive brines tend to support the conclusions of Orla-Jensen. It should be stressed, however, that determination of the salt tolerance of pure cultures of lactic acid bacteria is difficult. Therefore, until further investigations completely substantiate this view, the conclusion that Manzanillo brines never contain *Lactobacillus brevis* or other heterofermentative lactobacilli must be tentative.

Table 5 summarizes the bacterial population trends alluded to above and shows the accompanying chemical changes as indicated by changes in total acidity and pH values. The data shown were collected from November, 1939, to November, 1940. The olives were Sevillanos of the Colossal size, treated almost two thirds of the way to the pit with 1.5 per cent NaOH solution. No attempt was made to build the strength of the salt brine to the original strength, but fresh brine (25° salometer) was added as needed. Sugar was not added at any time during the fermentation.

Methods for Control of the Fermentation.—As already stressed, olives are sometimes difficult to ferment. All processors are cognizant of the difficulties encountered with the Manzanillo variety and realize that if the fermentation is not controlled properly, the olives do not cure, but deteriorate or even spoil completely.

Control of the fermentation of olives has been an important practical problem for many years. Cruess (1930) has devoted some time to control measures; his recommendations have included incubation (use of artificial heat to accelerate the fermentation), addition of supplementary fermentable material, and use of "starters."

Control of the fermentation is accomplished by proper manipulation of the biological, physical, and chemical factors which affect the activity

of the desirable lactic acid bacteria. For example, incubation is used to maintain fermentation during the winter months when otherwise it would remain dormant; supplementary sugar is added either to make up for the fermentable material lost by lye treatment and washing or

TABLE 5
GROSS FLORAL AND CHEMICAL CHANGES IN BRINE FROM A SEVILLANO FERMENTATION

Time in days	Approximate numbers of microorganisms per milliliter of brine		Total acidity, grams lactic acid per 100 milliliters brine	pH	Grams NaCl per 100 milliliters brine	Most abundant bacteria or yeasts isolated during the stages of fermentation
	Gram-positive	Gram-negative				
Primary stage						
0	43	254	0.0	8.20	6.25	Gram-negative bacteria
1	1,500	3,050	0.14	6.80		<i>Aerobacter</i> and <i>Pseudomonas</i>
2	6,890,000		0.37	6.35		
3	120,500,000	13,500,000	0.54	5.75	3.04	Gram-positive bacteria
5	390,000,000	228,500,000	1.08	5.20		<i>Streptococcus</i> and <i>Leuconostoc</i>
7	237,000,000	27,600,000	0.153	5.00		A few yeasts
Intermediate stage						
9	707,000,000	22,000,000	0.153	4.65		Gram-negative bacteria
11			0.24	4.30		<i>Aerobacter</i>
12	410,000,000	2,950,000	1.98	4.50	3.28	Gram-positive bacteria
15	31,000,000	0	2.43	4.35	3.33	<i>Leuconostoc</i> and <i>Lactobacillus</i>
18	126,000,000	0	2.97	4.40	3.51	
21	140,000,000	0	0.333	4.35	3.63	No yeasts
Final stage						
28		0	0.423	4.22	3.69	Gram-positive bacteria
35	14,000,000	0	4.05	4.20	3.69	<i>Lactobacillus plantarum</i>
42	3,500,000	0	4.14	4.05	3.86	<i>Lactobacillus brevis</i>
56	4,500,000	0	4.45	4.20	3.91	No Gram-negative bacteria or yeasts
77	1,500,000	0	4.32	4.20	3.83	
196	700,000	0	4.14	4.10	4.09	
365	2,650,000	0	0.514	4.50	4.09	

to replenish that lost through improper fermentation; and starters are used to add desirable lactic acid bacteria in such numbers that they control all other microbial competition for the fermentable substances in the brines.

ACCELERATION OF FERMENTATION BY INCUBATION

The fermentation of any variety of green olives may be accelerated by proper incubation. Cruess (1930) has studied some of the aspects of incubation of fermenting olives in detail but recommended against incubation of the whole pack of green fruit unless all of it was required

for early delivery. Commercial-scale experiments indicated that an average temperature of 75° F (23.9° C) was satisfactory for acceleration of acid production without impairing the quality of the pickled olives. However, as stressed by Cruess, the extra cost and care required for artificial heating of the barrels in incubation rooms militates against incubating all of the olives.

Commercial use of incubation has been successful to accelerate acid formation in fermenting green olives; but there has been much apprehension concerning the use of artificial heating, particularly with respect to the temperature which should be used, and the interval of time which should elapse after barreling before applying heat.

In order to firmly establish the optimum incubation temperature to be recommended for obtaining best results, it was necessary to determine the optimum temperature for acid production by the desirable lactic acid bacteria as well as the temperature range for maximum activity of the common spoilage bacteria.

The period of time which should elapse after barreling the fruit before starting the incubation was determined by making a detailed study of the persistence of the spoilage bacteria during the primary stage and first phase of the intermediate stage of the fermentation.

The test bacteria included all of the lactic acid bacteria isolated from various spontaneous fermentations which had been studied, as well as the spoilage bacteria obtained from many samples of defective olives.

Optimum Temperature for Acid Production by the Lactic Acid Bacteria.—For the different lactic acid bacteria, optimum temperature determinations were made by inoculation of each culture into 10-milliliter portions of yeast-infusion broth containing 2.0 per cent glucose, which had been adjusted to pH 7.0 before sterilization. One of each of the inoculated tubes of yeast-infusion dextrose medium was incubated at 19°, 25°, 30°, 34°, 37°, 40°, and 45° C (66.2°, 77.0°, 86.0°, 93.2°, 98.6°, 104.0°, and 113.0° F) for 1 week when the amount of total acid produced was determined by titration with 0.1N NaOH. Longer incubation of the cultures grown in the yeast-infusion dextrose medium did not give sufficiently higher total acid titrations to warrant consideration.

The results of these experiments are summarized in following figures and tables, which have been prepared to show the optimum temperature range for maximum acid production by the different species of lactic acid bacteria, as well as important variations in maximum acid production by different types of the various predominating species studied.

As already shown, the predominating lactic acid bacteria are *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Lactobacillus*

brevis. Leuconostoc mesenteroides is found only during the early stages of the fermentation and is the predominating organism during the primary stage and most of the intermediate stage. *Leuconostoc mesenteroides* has an optimum temperature for acid production from glucose that ranges between 19° C (66.2° F) and 30° C (86.0° F). Although the cultures tested were still active at 34° C (93.2° F) their ability to produce acid was markedly influenced at temperatures above 34° C (93.2° F) as is shown in table 6.

TABLE 6
OPTIMUM TEMPERATURE FOR ACID PRODUCTION FROM GLUCOSE BY
Leuconostoc mesenteroides

Milliliters of 0.1N NaOH to neutralize 10 milliliters culture	Number of cultures producing acid from glucose at various temperatures*						
	19° C	25° C	30° C	34° C	37° C	40° C	45° C
0.00-0.49.....	0	0	0	0	0	51	17
0.50-0.99.....	0	0	0	0	0	60	0
1.00-1.49.....	0	0	0	0	0	12	0
1.50-1.99.....	0	0	0	0	0	8	0
2.00-2.49.....	0	0	0	0	11	3	0
2.50-2.99.....	0	0	0	3	43	2	0
3.00-3.49.....	1	13	16	31	38	0	0
3.50-3.99.....	31	93	51	63	42	0	0
4.00-4.49.....	80	14	55	38	3	0	0
4.50-4.99.....	8	16	15	2	0	0	0
5.00-5.49.....	13	0	0	0	0	0	0
5.50-5.99.....	4	0	0	0	0	0	0
Total	137	136†	137	137	137	136‡	17
Mean.....	4.30	3.87	4.00	3.76	3.19	0.73	0.0‡

* All test cultures incubated for 7 days.

† One culture lost.

‡ None of the cultures tested were able to grow at 45° C.

Four types of *Leuconostoc mesenteroides* were found among the 137 cultures tested. Curves to show the characteristic activity of these types are shown in figure 10. The average amount of acid produced by all cultures grown at the various temperatures is also shown; this mean curve corresponds favorably with the results previously reported by Hucker and Pederson (1930).

Lactobacillus plantarum is the predominating species during the last part of the intermediate stage and all of the final stage of fermentation. This species has an optimum temperature for acid production from glucose that ranges between 30° C (86.0° F) and 37° C (98.6° F). Considerable variation was encountered among the 197 cultures which were examined, as is to be seen by perusal of table 7 and figure 11; four general types of *L. plantarum* were encountered as is shown in the figure. The

average temperature range for maximum acid production by all of the cultures tested at the various temperatures corresponds well with those previously reported by Pederson (1936). However, as is clearly seen

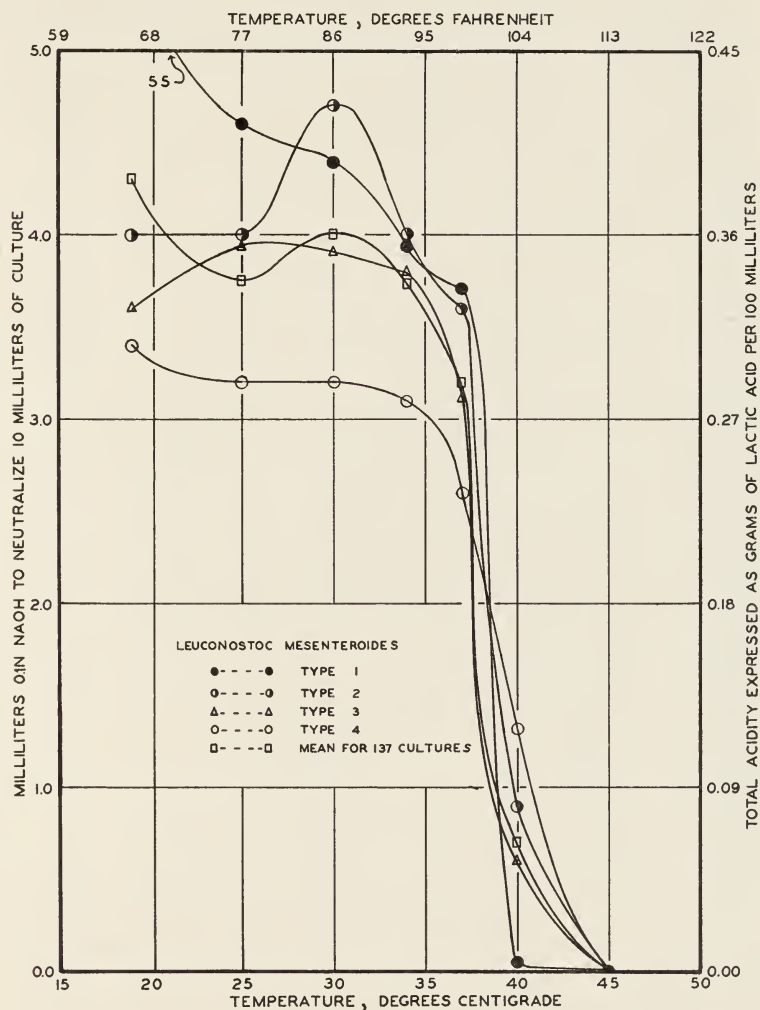


Fig. 10.—Optimum temperature for acid production from glucose by types of *Leuconostoc mesenteroides*.

by examination of the data in table 7, and summarized in figure 11, the optimum temperature varies between 30° C (86.0° F) and 37° C (98.6° F), under the conditions of these experiments.

Lactobacillus brevis is also of importance throughout the final stage of fermentation although, from all indications, it is not present in as large numbers as is *L. plantarum*. Marked variation in optimum tem-

TABLE 7
OPTIMUM TEMPERATURE FOR ACID PRODUCTION FROM GLUCOSE BY
Lactobacillus plantarum

Milliliters of 0.1N NaOH to neutralize 10 milliliters culture	Number of cultures producing acid from glucose at various temperatures						
	19° C	25° C	30° C	34° C	37° C	40° C	45° C
0.00- 0.49	0	0	0	0	0	13	54
0.50- 0.99	0	0	0	0	0	0	7
1.00- 1.49	0	0	0	0	0	0	0
1.50- 1.99	0	0	0	0	0	1	0
2.00- 2.49	0	0	0	0	0	1	0
2.50- 2.99	0	0	0	0	0	6	1
3.00- 3.49	0	1	0	1	1	1	2
3.50- 3.99	1	0	0	0	1	1	0
4.00- 4.49	3	0	0	0	1	5	1
4.50- 4.99	3	1	0	0	0	4	2
5.00- 5.49	11	0	1	0	1	6	4
5.50- 5.99	24	0	0	1	0	14	0
6.00- 6.49	42	3	0	2	4	10	4
6.50- 6.99	33	10	1	2	2	26	4
7.00- 7.49	21	34	6	6	10	33	4
7.50- 7.99	20	32	13	8	15	11	4
8.00- 8.49	31	27	25	17	48	9	3
8.50- 8.99	6	17	25	41	26	6	1
9.00- 9.49	1	20	27	28	9	15	2
9.50- 9.99	1	27	11	12	2	21	1
10.00-10.49	0	12	16	9	5	7	1
10.50-10.99	0	3	32	10	21	2	0
11.00-11.49	0	5	14	33	31	1	0
11.50-11.99	0	1	10	13	10	3	0
12.00-12.49	0	0	4	5	2	0	0
12.50-12.99	0	3	5	1	0	0	0
13.00-13.49	0	0	2	1	1	0	0
13.50-13.99	0	0	2	3	1	0	0
14.00-14.49	0	0	1	1	3	0	1
14.50-14.99	0	0	1	0	0	1	0
15.00-15.49	0	0	0	1	2	0	0
15.50-15.99	0	0	1	1	0	0	0
16.00-16.49	0	0	0	0	0	0	0
16.50-16.99	0	0	0	1	0	0	0
17.00-17.49	0	0	0	0	0	0	0
17.50-17.99	0	0	0	0	1	0	0
Total	197	196*	197	197	197	197	96
Mean	6.83	8.52	9.81	9.80	9.42	6.96	2.71

* One culture lost.

perature for acid production from glucose was also observed among the 55 cultures of *L. brevis*. According to Pederson (1938), the optimum temperature range for this species is from 28° C (82.4° F) to 32° C (89.6° F). With the yeast-infusion dextrose broth used in these experiments, the temperature range for maximum acid production by the various cultures of this species varied between 30° C (86.0° F) and 37° C (98.6° F). Particular attention is called to this difference, for in

all other respects the cultures corresponded almost perfectly with Peder-son's description of *L. brevis*. The optimum temperature for maximum acid production was found to be 34° C (table 8). The variations en-counterred with different strains of *L. brevis* are shown in figure 12.

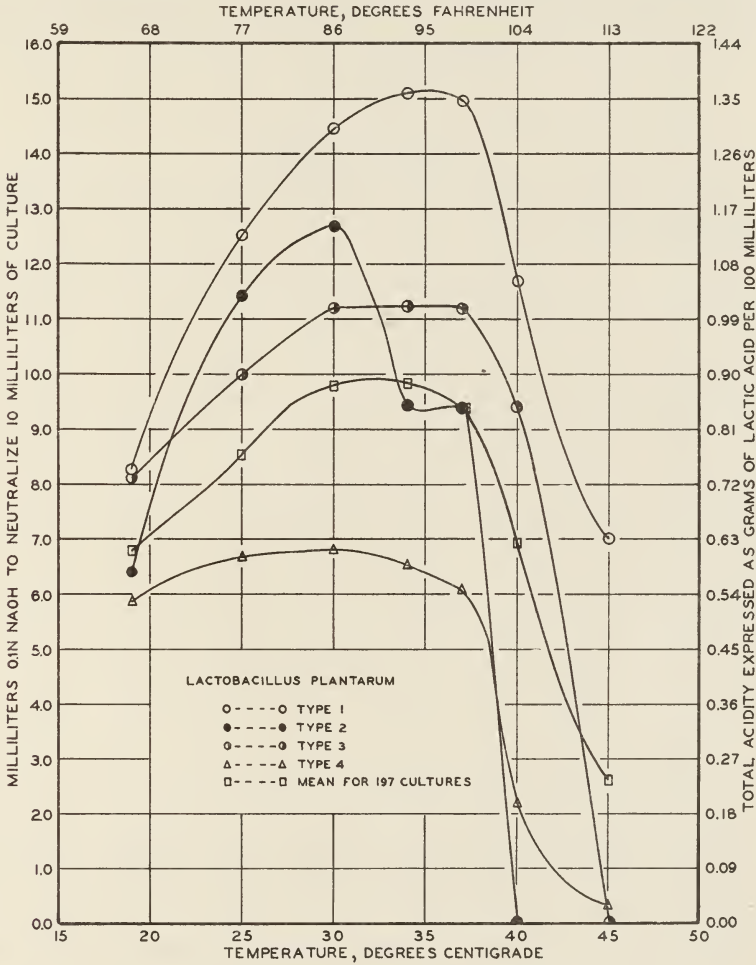


Fig. 11.—Optimum temperature for acid production from glucose by types of *Lactobacillus plantarum*.

In addition to the above three species which have been shown to pre-dominate in the normal fermentation of olives, fourteen other gas-forming cultures were isolated. Eight of these cultures were identified as *Lactobacillus buchneri*. The remaining six cultures were identified as *Lactobacillus fermenti*. The optimum temperature ranges for acid pro-duction from glucose by these two species corresponded well to the optimum temperatures recorded by Pederson (1938).

Thus it is seen that the optimum temperature for acid production from glucose ranges between about 20° C (68.0° F) and 37° C (98.6° F), according to the species and the individual variation of the different isolates of lactic acid bacteria.

Cruess (1930) has found, from commercial-scale studies, that a temperature range of from about 70° to 75° F (about 21.1° to 23.9° C)

TABLE 8
OPTIMUM TEMPERATURE FOR ACID PRODUCTION FROM GLUCOSE BY
Lactobacillus brevis

Milliliters of 0.1N NaOH to neutralize 10 milliliters culture	Number of cultures producing acid from glucose at various temperatures						
	19° C	25° C	30° C	34° C	37° C	40° C	45° C
0.00-0.49	0	0	0	0	0	0	12
0.50-0.99	0	0	0	0	0	0	0
1.00-1.49	0	0	0	0	0	0	0
1.50-1.99	3	0	0	0	0	1	3
2.00-2.49	4	3	0	0	1	0	2
2.50-2.99	7	3	4	3	2	4	3
3.00-3.49	18	5	1	2	2	6	2
3.50-3.99	8	14	9	3	14	12	5
4.00-4.49	5	13	13	18	15	16	3
4.50-4.99	6	3	9	12	3	1	2
5.00-5.49	4	3	5	0	1	6	8
5.50-5.99	0	3	3	4	6	9	2
6.00-6.49	0	6	5	6	8	1	0
6.50-6.99	0	2	4	3	1	0	0
7.00-7.49	0	0	2	4	2	0	0
Total	55	55	55	55	55	55	47
Mean	3.50	4.31	4.77	4.90	4.65	4.28	2.94

gave very satisfactory pickled olives. As is seen from the above experiments, this temperature range is optimum for the activity of *Leuconostoc mesenteroides* types, which are the first lactic acid bacteria to predominate in the desirable bacterial population. Since temperatures much above 90° F have been observed to be undesirable for pickling, the recommendations of Cruess can be followed with assurance. The maximum temperature should not exceed 85° F (29.4° C), for spoilage may result. In addition, at temperatures between about 85° and 100° F, the spoilage bacteria are generally at their maximum activity provided other conditions are optimum for their growth. Furthermore, in common with other microbial fermentations, it is recognized that a slower fermentation generally results in a pickle of better quality.

Optimum Temperatures for Spoilage Bacteria.—The most common bacteria causing deterioration of olives during the initial stages of fermentation are the coliform bacteria (species of *Aerobacter* and *Escher-*

ichia) and the butyric acid bacteria (*Clostridium butyricum* and other species). All of these bacteria grow exceedingly well in olive brines at temperatures ranging between 86° and 104° F (30° to 40° C); and even at 77° F (25° C), growth is almost maximum. Thus it is obvious that artificial heating should not be applied until one has determined that

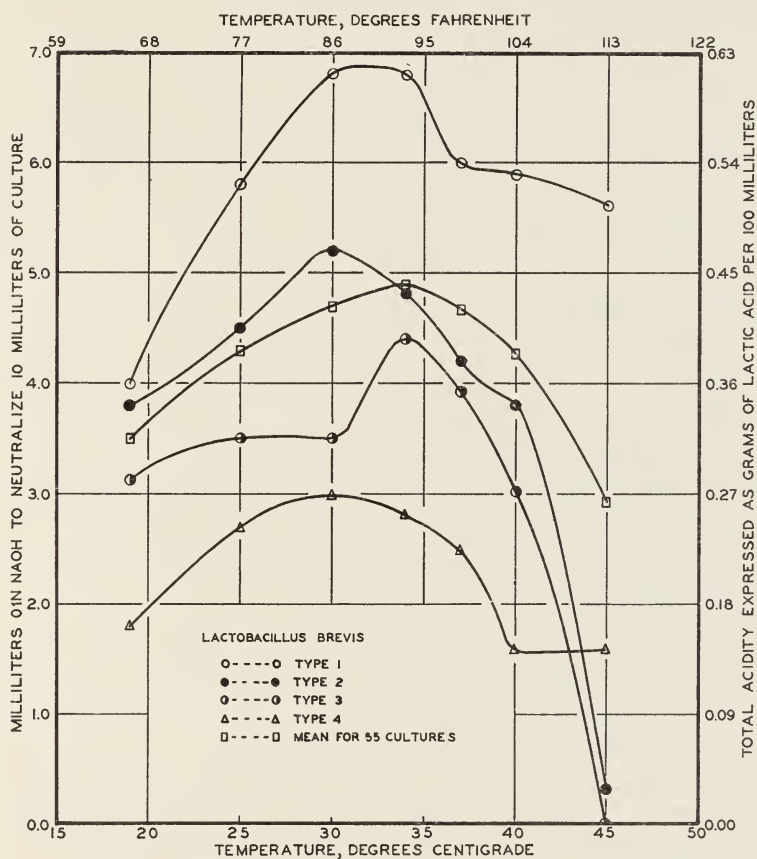


Fig. 12.—Optimum temperature for acid production from glucose by types of *Lactobacillus brevis*.

these spoilage bacteria (which are probably always present at the beginning of the fermentation) have been eliminated. With fermentations which proceed in a normal manner the time will vary between 1 and 3 weeks according to the activity of the lactic acid bacteria. In abnormal Manzanillo fermentations, on the other hand, spoilage bacteria have been observed to persist for periods up to 3 or 4 months, and finally to completely spoil the olives.

Two aids in determining the time which must elapse before the bar-

reled, brined olives are safe for incubation include microscopical examination and determination of the pH of the fermenting brines. Most of the spoilage bacteria mentioned above are motile. Only a few *Clostridium butyricum* types and practically none of the coliform bacteria can multiply in brines having a pH of 4.5 or less, provided that the lactic acid bacteria are still active and the salt content of the brines is between 7.0 and 7.5 per cent.

Effect of Supplementary Sugar on the Fermentation.—Experiments made by Cruess (1930) have shown conclusively that it is necessary to add supplementary fermentable material in order to obtain satisfactory acid production in California green olives. Additional commercial experience coupled with laboratory observations has resulted in recognition of the fact that for the best-quality olives, corn sugar should be added to all Manzanillo brines and to those Sevillano brines which do not attain a satisfactory acid content. The flavor and keeping quality of green olives is dependent upon the acidity produced by fermentation. Furthermore, it is recognized that the natural acidity (principally lactic acid with some acetic acid) produced by the desirable bacteria is essential for superior color as well as flavor.

The chief problem connected with control of the fermentation has been concerned with the time interval which must elapse after the olives have been barreled and brined before it is safe to add sugar; if the supplementary sugar is added immediately after brining, spoiling of the olives very often occurs.

Supplementary fermentable material may be added with assurance only when the undesirable bacteria have been eliminated. As already stated, the spoilage bacteria are rendered inactive or completely eliminated from normal fermentations in from about 1 to 3 weeks, but in abnormal fermentations they may persist indefinitely. Here, too, laboratory control is necessary; and the same tests used to determine the time which must elapse before incubation should be used for safe control of the fermentation.

Cruess (1930) found that addition of commercial glucose sugar (Cerelese) at the rate of 2 pounds per barrel was the most satisfactory procedure. In the industry, glucose is commonly added at the rate of 1 to 2 pounds per barrel. Several additions are sometimes necessary before a satisfactory acidity has been formed in the brines. No particular agreement exists in the industry as to what constitutes a desirable acidity, but for satisfactory storage the brines must be at about pH 4.0.

The effect of supplementary sugar on the fermentation as indicated by increased acid production is clearly shown in figure 13. Qualitative tests for the presence of reducing sugar in the brines should be made in

order to follow the course of fermentation of the added sugar. When no sugar remains, more is added until the desirable pH has been attained.

"STUCK" FERMENTATIONS

"Stuck" fermentations commonly occur with Manzanillo olives. In such instances repeated additions of sugar are depleted without any

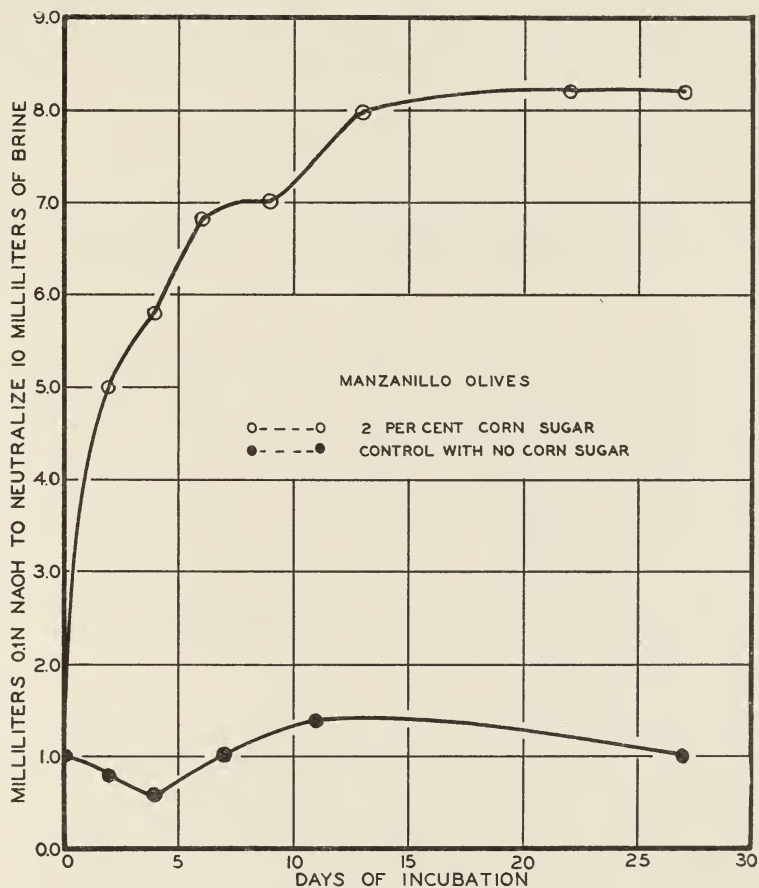


Fig. 13.—Effect of addition of corn sugar on acid production in brine, with Manzanillo olives.

significant increase in acid production. Such fermentations are wasteful and control is necessary to avoid loss of olives.

As the "stuck" olive fermentations result when yeasts predominate the fermentation to the exclusion or inactivation of the desirable lactic acid bacteria, repeated additions of sugar only aggravate the problem. Proper control of these fermentations consists of replacing the undesirable yeast population with desirable lactic acid bacteria. This may

be accomplished satisfactorily by addition of normal, actively fermenting brine in concentrations of 10 to 20 per cent, or in extreme cases by removal of the abnormal brine and replacement with fresh brine which has been heavily inoculated with normal, actively fermenting brine, to insure a seeding of desirable bacteria.

Fortunately, experimental Manzanillo fermentations became "stuck" in the laboratory and it was possible to ascertain that yeasts were responsible for the abnormal fermentations. For a period of 7 weeks

TABLE 9
FLORAL AND CHEMICAL CHANGES IN BRINE FROM A "STUCK"
MANZANILLO FERMENTATION*

Time in days	Yeasts per milliliter of brine	Acidity as grams lactic per 100 milliliters brine	pH	NaCl as grams per 100 milliliters brine
0	8	0.009	8.2	5.8
1	7	.054	7.5	4.1
2	4,380	.081	7.2	3.9
3	19,700	.063	7.1	3.8
7	155,000	.112	6.5	3.8
9	975,000	.090	6.7	3.5
16	No count	.072	6.6	4.1
21	245,000	.144	5.6	3.9
28†	255,000	.090	5.6	3.9
35	1,450,000	.153	5.6	3.9
42†	10,250,000	.186	5.6	3.9
49†	2,550,000	.225	5.8	4.0
56†	14,550,000	0.126	5.2	4.0

* Methods and media used identical with study of biological and chemical changes occurring in normal fermentations. No Gram-positive or Gram-negative bacteria were isolated or seen in microscopic preparations until inoculations were made.

† On these days 0.5 per cent glucose was added.

bacteria were never isolated from these brines or seen in the frequent microscopical examinations. Microbiological and chemical changes occurring in these abnormal fermentations are shown in table 9.

At the end of 7 weeks, lactic acid bacteria still were absent and starters of normal, actively fermenting brine and more sugar were added. After 1 more month, during which there were four separate additions of starter (1 to 10 per cent by volume) and two additions of sugar, the pH had decreased to 4.6 and desirable lactic acid bacteria were found.

"Stuck" Manzanillo fermentations are frequently noticed because of failure to recognize that this variety, low in fermentable substances in the fresh state, loses a large proportion of its fermentable material during lye treatment and washing and that the amount of sugar and other fermentable compounds remaining is not sufficient to support continued good growth of the desirable bacteria.

"Stuck" fermentations can be treated successfully without appreci-

able loss of quality of the finished pickles if proper control measures are taken soon enough. On the other hand, if the olives are allowed to go through the winter without treatment, losses are high through bacterial decomposition; such fermentations should be treated as soon as they are detected.

Complete understanding of the problem of microbial species associations and competition of the various microorganisms for utilizable substrates in green-olive brines is of utmost importance to insure satisfactory fermentations. Unfortunately, at present, little is known of these phenomena with respect to olive fermentations. Until adequate study of the microbial populations found during the primary stage of the fermentation has been made, it is impossible to say that yeasts are the only microorganisms which may be responsible for "stuck" fermentations in which sugar is used up without accumulation of lactic acid in the brines.

CONTROL OF THE FERMENTATION BY THE USE OF STARTERS

The principle of pure-culture inoculation involves mass transfer of pure cultures of the desired microorganism in numbers sufficient to insure domination of the fermentation, which excludes the spoilage types through competition for the fermentable material. The pure-culture technique, first adopted for yeasts in the brewing and wine industries, has found wide application in the dairy industry and in the industrial-fermentations industry, where a host of different bacteria, yeasts, and molds are used.

Initiation of the lactic acid fermentation in green-olive brines depends upon the presence of lactic acid bacteria. Cruess and Guthrie (1923) reported results which showed that lye treatment destroyed most of the microorganisms present on the olives at the time of treatment. If the lactic acid bacteria have been destroyed by the action of the lye and none gain access to the olives in appreciable numbers during subsequent washing, barreling, and brining it is obvious that the desirable fermentation will not take place. Furthermore, if undesirable bacteria are present in the wash water or brine, the olives very frequently spoil. To obviate such a situation, Cruess (1930) recommended the use of starters of normal brine. Later (1937) he suggested the use of pure cultures of lactic acid bacteria for starting green-olive fermentations. Since 1937 pure-culture inoculations have been used extensively in the industry.

Initiation and maintenance of the fermentation are of primary importance for production of highest-quality green olives. They are important particularly in connection with pickling the Manzanillo olive, for

reasons already stressed. Thus it is seen that development of a completely successful inoculation technique to be used in the pickling of green olives is of primary importance.

Since the study of the microbiology of green olives was first started in 1937, numerous opportunities to study the effect of pure-culture starters on Manzanillo and Sevillano commercial fermentations have arisen. Use of pure-culture inoculations have been successful in the laboratory and on an industrial scale. To date, however, starters have been most successful for accelerating the fermentation of Sevillano fruit. Results with Manzanillo olives have not been so striking but have given satisfactory results in the majority of the experiments.

Preparation of Pure-Culture Starters.—To be of any great value pure-culture starters must of necessity be inexpensive, simple to prepare, easy to use, and sure to give satisfactory results. For use in inoculation, either of laboratory or of commercial fermentations, the medium finally chosen after some experimental trials consisted of one part of tomato juice and one part of water to which enough salt was added to give a final concentration of 3.0 per cent salt. The finished medium, contained in glass or in olive-oil tins, was sterilized by steam under pressure or by repeated pasteurization.

In the early experiments the solids were removed from the tomato juice by filtration. It was soon found practicable to use unfiltered tomato juice and filtration was discontinued. The starter medium was inoculated by introduction of heavy seedings of the different lactic acid bacteria studied. The inoculated starter was incubated at room temperature for 2 to 4 days before being used to seed the olive brines.

Selection of Cultures.—The pure cultures used by Cruess (1937) included the gas-forming rod-shaped species *Lactobacillus pentoaceticus*, *L. gayonii*, and *L. mannitopeus*, which are synonyms of *L. brevis*, *L. fermenti*, and *L. buchneri*, respectively; the non-gas-forming rod *L. pentosus*, correctly known as *L. plantarum*; and *Leuconostoc mesenteroides*, a gas-forming, spherical bacterium. *Lactobacillus plantarum* (*L. pentosus*) was considered to be the most promising of these cultures.

The *pentosus* strain has been used extensively in inoculation experiments conducted since 1937, and has been compared with various cultures which have been isolated from the brines of fermenting olives. None of the strains of *Lactobacillus plantarum* differed significantly in their effect on the acceleration of the fermentation, nor was it possible to detect appreciable differences in quality of the olives pickled in brines which had been inoculated with the different cultures.

The gas-forming, rod-shaped lactic acid species *Lactobacillus brevis*, *L. buchneri*, and *L. fermenti* isolated from olives were not particularly

useful for starters. As already seen, they do not produce as much acid as the homofermentative species, *L. plantarum*; they are not as salt-tolerant, and very often are difficult to grow. Furthermore, they are wasteful of the sugar inasmuch as a part of the glucose is decomposed with the formation of carbon dioxide gas.

Tests made with *Leuconostoc mesenteroides*, the gas-forming, spherical species dominating the early stages of the normal fermentation, were satisfactory under laboratory and industrial conditions when Sevillano fruit was used. This organism, however, plays no part in completion of the olive fermentation and is subject to the same objections as the gas-forming lactobacilli. It is suggested that starters for green-olive fermentations be prepared only with *Lactobacillus plantarum*.

Size of Inoculum.—A series of experiments conducted on a commercial scale, in coöperation with different picklers, indicated that 2 quarts of fresh starter per 50-gallon barrel gave satisfactory results. Larger quantities of starter (up to 1 gallon) did not give sufficiently greater acceleration of the fermentation to warrant routine inoculation of the entire pack with 1 gallon of starter per barrel. Laboratory-scale experiments using corresponding amounts of starter added to 5-gallon oak kegs of green olives gave results corresponding to those obtained under commercial conditions.

Under conditions existing in "stuck" fermentations a much larger pure-culture starter is often desirable. In case of incipient spoilage a heavy seeding with a pure-culture starter is often beneficial. For either type of abnormal fermentation, as well as cases where the lactic acid fermentation has not started, it is equally satisfactory to replace large portions or all of the abnormal brine either with a pure-culture starter or with normal, actively fermenting brine taken from other olives.

It is to be understood that the olive technologist must have had some bacteriological training if pure-culture starters are to be used with safety. Otherwise, use of normal, actively fermenting brines is mandatory.

Factors Influencing the Acceleration of Fermentation with Starters.—Acceleration of the fermentation through the use of starters is of particular value in controlling the undesirable bacteria present during the primary stage of fermentation. The effect of inoculation is most noticeable on the rate of acid production and on decrease in the pH value during the first 2 months of the fermentation period. Sevillano olives respond to inoculation particularly well as is shown in table 10.

This experiment, conducted during the 1940-1941 season, showed that fermentation was accelerated by addition of starter and, in this case, gas-pocket spoilage was suppressed. However, there are several factors

which influence the efficiency of starters; and it should be stressed that mere addition of a starter of a pure culture of a desirable lactic acid bacterium or normal, actively fermenting brine to olives may not be enough to initiate fermentation.

Some of the factors which are known to significantly influence the acceleration of fermentation by the use of starters are:

1. The amount of fermentable material (glucose and mannitol) available.

TABLE 10
EFFECT OF PURE-CULTURE INOCULATION ON RATE OF FERMENTATION
OF SEVILLANO OLIVES*

Date	Fermentation as indicated by decrease of pH†		Fermentation as indicated by increase in total acidity‡	
	Inoculated (8 barrels)	Controls (7 barrels)	Inoculated (8 barrels)	Controls (7 barrels)
Oct. 10, 1940§	6.03	6.06	0.121	0.103
Oct. 18, 1940	4.89	5.36¶	.244	.181
Oct. 25, 1940	4.50	4.88	.378	.267
Nov. 4, 1940	4.33	4.63	.460	.336
Nov. 16, 1940	4.29	4.46	.476	.399
Dec. 3, 1940	4.23	4.48	.538	.432
Jan. 6, 1941	4.16	4.23	.533	.442
Feb. 28, 1941	4.08	4.13	.678	.609
May 27, 1941	3.73	3.84	0.684	0.610

* Olives were Jumbo size, treated halfway to the pit with lye, washed to about pH 8.2, and barreled 2 days prior to inoculation. No sugar was added during the course of the fermentation.

† pH is expressed as the average for each series of barrels.

‡ Total acidity is expressed in grams of lactic acid per 100 milliliters of brine as the average for each series.

§ Samples taken just before inoculations were made.

¶ Gas pockets were found in the uninoculated olives.

2. The concentration of salt in the brine.

3. The interval of time elapsing between brining of the olives and inoculation of the brine.

4. The availability of necessary growth-promoting substances (bacterial vitamins) for the starter culture.

5. The effect of acidified brines on the starter culture.

6. The effect of temperature on the starter culture.

As already stressed, availability of fermentable compounds, as influenced by lye treatment and washing, determines in part the fermentability of olives. Ordinarily, the Sevillano olive responds rapidly to inoculation alone because more fermentable material remains at the time of brining. On the other hand, Manzanillo fruit may show little response to inoculation unless supplementary fermentable material is furnished for the added bacteria. This effect on inoculation is shown in figure 14. This experiment, one of several, was conducted with Large

Manzanillo fruit, treated about three fourths of the way to the pit with lye, washed to pH 8.0, barreled in 5-gallon kegs, and covered with a brine of 60° salometer. It is readily seen that inoculation alone had little effect on acceleration of acid production. However, when corn sugar was made available, acid production increased significantly. *This is further proof that inoculation alone is frequently not sufficient to accelerate acid production.*

Another experiment testing the effect of the addition of sugar under various conditions is summarized in figure 13. This experiment was conducted with Extra Large Manzanillo fruit, treated about three

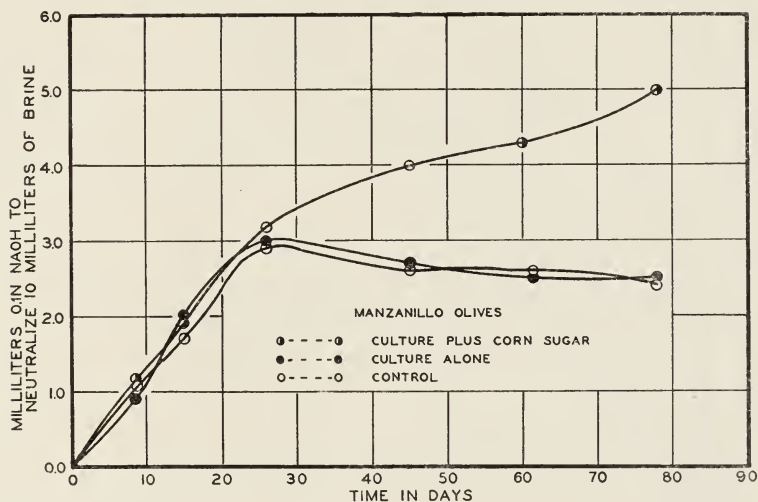


Fig. 14.—Effect of addition of culture and corn sugar on acid production in brine, with Manzanillo olives.

fourths of the way to the pit with lye, washed to pH 7.8, barreled in 5-gallon kegs, covered with a brine of 60° salometer, and allowed to stand 1 week at room temperature before making the additions of sugar and starter of *Lactobacillus plantarum* and actively fermenting brine. As is seen by examination of the curves shown in figure 15, the addition of sugar results in a definite, desirable increase in the amount of acid produced. In this particular experiment it is interesting to note that sugar alone stimulated acid production markedly. However, it should be stressed again that, unless one knows that the brines contain a satisfactory population of desirable lactic acid bacteria, the addition of sugar without a simultaneous inoculation with a pure-culture starter or normal, actively fermenting brine may result in loss of the olives by spoilage bacteria or loss of sugar due to the activity of yeasts which decompose the sugar without appreciably increasing the acidity of the brine (see the section “‘Stuck’ Fermentations”).

It has been known for some time that certain of the lactic acid bacteria need growth factors (bacterial vitamins) in order to attain their maximum activity. One might assume that the Manzanillo olive is lacking in the necessary growth-promoting substances, since it is often difficult to ferment properly. Tomato juice has been used extensively to stimulate growth of the lactic acid bacteria; and, since it has always been a con-

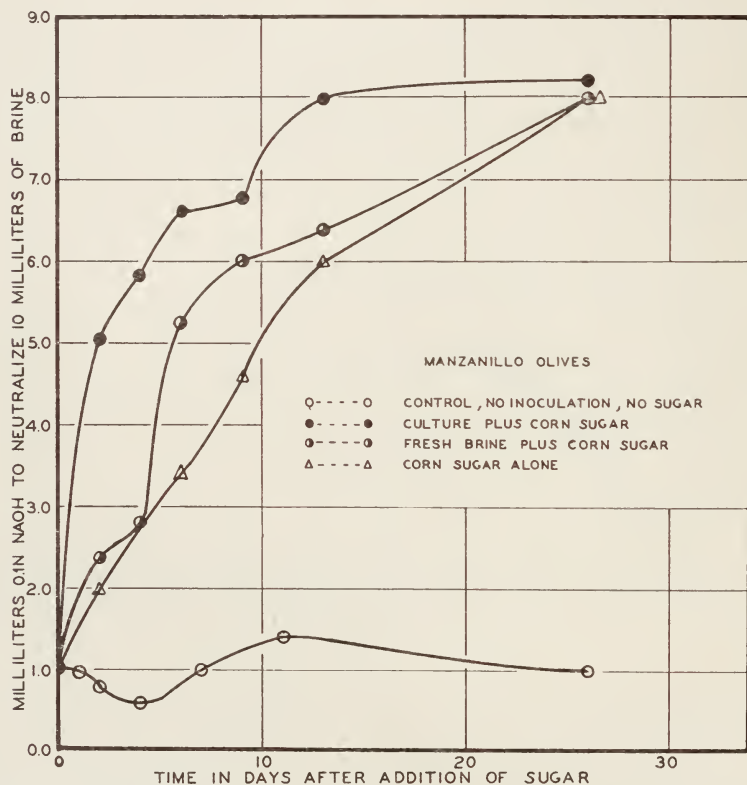


Fig. 15.—Effect of addition of corn sugar, culture, and fresh brine starter on acid production in brine, with Manzanillo olives.

stituent of all pure-culture starters, there was hope that the mere addition of tomato juice would accelerate the acid production in fermenting Manzanillo olives. The experiment summarized in figure 16 shows that with Manzanillo olives, the addition of tomato juice alone had no more effect on acceleration of acid production than did the same quantity of pure-culture starter. This experiment was conducted with Large Manzanillo olives treated from one half to two thirds to the pit with lye, washed to pH 8.0, put up in half-gallon glass jars, covered with a brine of 55° salometer, and treated immediately. Two jars

were inoculated with a culture of *Lactobacillus plantarum* (at the rate of 2 quarts per 50-gallon barrel). Two other jars were inoculated with the same species and the brines supplemented with 2 per cent corn sugar. Two more jars received tomato juice at the rate of 2 quarts per 50-gallon barrel. Two additional jars received tomato juice and Cerelese at the same rate and the three remaining jars were left untreated for controls. The results shown in figure 16 are averages. Additional laboratory and field experiments conducted with Sevillano and Manzanillo olives have given comparable results.

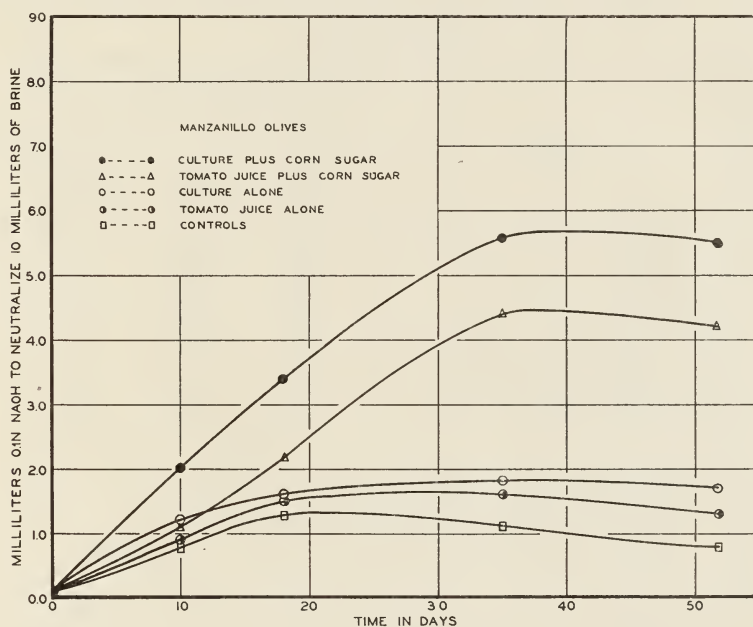


Fig. 16.—Effect of addition of corn sugar, tomato juice, and culture on acid production in brine, with Manzanillo olives.

Further experiments with other compounds thought to be of value in stimulating growth of the desirable lactic acid bacteria have been conducted. The substances which have been studied include yeast autolysate, yeast infusion, Tryptone, and the rice-bran-concentrate Galen B. Thus far results of these experiments have been inconclusive. On the basis of observations made since 1937 it seems probable that sufficient growth-promoting substances are available in the brines, provided sufficient fermentable material is available at the same time. This supposition is borne out by consideration of the above-mentioned experiments dealing with the use of starters and the addition of supplementary glucose to the brines. It is to be remembered however, that unless the brine is seeded with the desirable bacteria, fermentation will not ensue.

ACIDIFICATION OF THE BRINE

Under conditions where there is danger of loss of fruit resulting from abnormal fermentation it frequently has been suggested that the abnormal brines be acidified. This procedure was first suggested by Cruess (1930). Some packers have at one time or another acidified the brines used for the olives at time of addition of the first brine to the barreled

TABLE 11
EFFECT OF ACIDIFICATION OF THE BRINE WITH ACETIC ACID,
ON ACID PRODUCTION*

Date	Mean acid production expressed as grams of lactic acid per 100 milliliters		
	Controls (4 barrels)	Inoculated† (2 barrels)	Inoculated and acidified‡§ (3 barrels)
Oct. 8, 1940†.....	0.045	0.058	0.408
Oct. 18, 1940.....	.138	.239	.214
Oct. 25, 1940.....	.189	.316	.245
Nov. 4, 1940.....	.238	.357	.334
Nov. 16, 1940.....	.243	.345	.355
Dec. 4, 1940.....	.250	.342	.306
Jan. 6, 1941.....	.252	.401	.363
Feb. 28, 1941.....	.329	.432	.441
May 27, 1941.....	0.320¶	0.401	0.404

* Sevillano olives were Mammoth size, treated about halfway to the pit with lye, washed to about pH 8.0, and barreled 2 days prior to inoculation.

† Samples taken immediately after treatment.

‡ One quart of *Lactobacillus plantarum* culture was added to each barrel.

§ One quart of 100-grain distilled vinegar was added to each barrel.

¶ One 50-gallon barrel spoiled with softening and butyric acid production.

fruit, believing that such treatment would minimize losses resulting from the activity of spoilage bacteria. The following experiments deal with the effect of acidification on rate of fermentation.

The results found in table 11 show, in part, the effect of acidification of the brine with acetic acid, on the rate of fermentation of Sevillano Mammoth size fruit treated about halfway to the pit with lye, washed to about pH 8.0, barreled in 50-gallon barrels, and covered with brine of 40° salometer. Three of the barrels of olives were inoculated with 1 quart of *Lactobacillus plantarum* culture for each barrel. Acetic acid was added to these three barrels in the form of 100-grain distilled vinegar at the rate of 1 quart of 100-grain vinegar per barrel of olives. Two additional barrels received only culture and four barrels were used as controls.

As expected, the initial effect of acidification was to increase the acidity of the brines; but during the course of the fermentation period the initial high acidity decreased, and in the long run the total acidity

of the inoculated brines equaled the acidity of those brines receiving the acetic acid and starter. The olives in this experiment were of satisfactory quality at the end of the fermentation period except for those of one control barrel, which were lost by softening and butyric acid production. No appreciable taste of the vinegar remained in the acidified

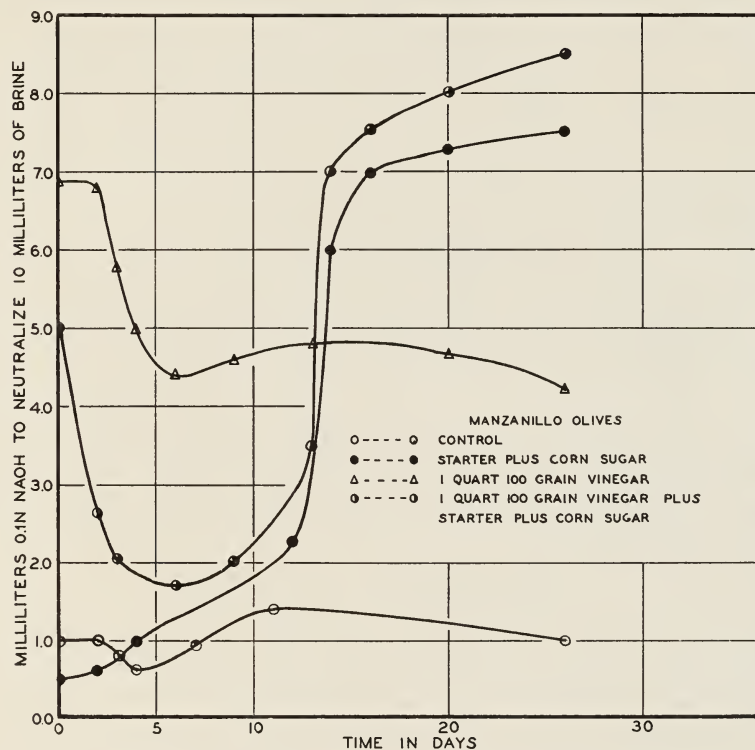


Fig. 17.—Effect of addition of vinegar on subsequent acid production in brine, with Manzanillo olives.

brines at the termination of the experiment, although it was noted that the acidified olives had a somewhat darker color than the plain inoculated or uninoculated olives.

Manzanillo olives do not always react favorably when fermented in acidified brines without further control of the fermentation, as is shown in figure 17. Here it is seen that acidification had no effect on subsequent acid production unless the acidified brine was fortified with 2 per cent corn sugar and inoculated with 1 quart of starter of *Lactobacillus plantarum* per 50-gallon barrel. These olives were Large Manzanillo fruit treated three fourths of the way to the pit with lye, washed to about pH 7.2, barreled in 50-gallon barrels, and covered with a

brine of 40° salometer. This experiment was unexpectedly terminated before observations of the effect of acidifying the brine on quality of the finished pickles were made.

TABLE 12
EFFECT OF ACIDIFICATION OF THE BRINE ON THE RATE OF ACID PRODUCTION
DURING FERMENTATION

Treatment	Num- ber of sam- ples	Mean acid content as grams lactic acid per 100 milliliters at various intervals of time after treatment								Final pH of brine
		1 day	2 days	3 days	5 days	13 days	30 days	47 days	63* days	
Uninoculated (controls)†	4	0 009	0 013	0 018	0 045	0 153	0 207	0 216	0 225	4 40
Inoculated with culture‡	2	.030	.036	.027	.063	.153	.225	.207	.207	4 50
Inoculated, plus 2 per cent sugar§	2	.036	.036	.018	.072	.207	.351	.414	.450	3 80
0.25 per cent acetic acid¶	2	.158	.113	.081	.090	.207	.378	.387	.397	4 10
0.50 per cent acetic acid	2	.405	.369	.324	.315	.351	.486	.495	.585	4 00
0.25 per cent lactic acid	2	.090	.059	.032	.063	.198	.315	.333	.387	4 00
0.50 per cent lactic acid	2	.236	.189	.126	.117	.189	.342	.360	.468	3 90
0.25 per cent hydrochloric acid	2	.153	.135	.108	.135	.207	.234	.216	.270	3 65
0.50 per cent hydrochloric acid	2	.374	.405	.360	.441	.468	.495	.468	.459	2 05
0.25 per cent sulfuric acid	2	.072	.081	.054	.081	.180	.252	.324	.333	3 75
0.50 per cent sulfuric acid	2	.189	.203	.144	.180	.270	.306	.297	.279	3 20
0.25 per cent phosphoric acid	2	.122	.207	.203	.288	.351	.378	.387	.306	4 20
0.50 per cent phosphoric acid	2	.279	.513	.509	.513	.504	.477	.504	.504	4 00
0.25 per cent acetic acid, cul- ture and sugar	2	.207	.144	.126	.180	.333	.630	.756	.828	3 60
0.50 per cent acetic acid, cul- ture and sugar	2	.485	.396	.387	.441	.432	.648	.846	.990	3 50
0.25 per cent lactic acid, cul- ture and sugar	2	.144	.117	.099	.144	.261	.495	.476	.585	3 65
0.50 per cent lactic acid, cul- ture and sugar	2	.284	.243	.162	.144	.270	.468	.540	.504	3 85
0.25 per cent phosphoric acid, culture and sugar	2	.311	.306	.234	.293	.351	.540	.594	.585	3 75
0.50 per cent phosphoric acid, culture and sugar	2	0 455	0 439	0 468	0 513	0 450	0 486	0 567	0 630	3 65

* Decreases in acid content due to yeast films.

† No treatment.

‡ Culture of *Lactobacillus* added at the rate of 2 quarts per 50-gallon barrel of olives.

§ Corn sugar (Cerelese) added to brine at rate of 2.0 per cent by volume.

¶ All acids added to brine on volume basis calculated in terms of 0.25 and 0.50 per cent lactic acid.

For some time acidification of silage has been recommended by Virtanen (1936, 1937) and others to prevent losses resulting from abnormal fermentations. In this process of preserving silage (called the A.I.V. process in honor of the originator, Dr. A. I. Virtanen) the silage is treated with an inorganic acid, such as sulfuric acid, to decrease the initial pH of the silage to such an extent that the butyric acid bacteria and other spoilage microorganisms cannot grow. It was thought that such a procedure might be of value for the preservation of olives, and

experiments were conducted to observe the effect of acidification of the brine with various organic and inorganic acids and to compare this procedure with the practice of using pure-culture starters and supplementary corn sugar. The results of one such experiment are shown in table 12. Acidification had a marked effect on the acidity of the brine during the primary stage of the fermentation. The effect was influenced by the concentration as well as the kind of acid added. Addition of the inorganic acids—hydrochloric, sulfuric, or phosphoric—exerted more effect on acidity of the brine than addition of either acetic or lactic acid. When acidification of the brine was accompanied by inoculation and addition of Cerelease, satisfactory fermentations were obtained with acetic and lactic acid. The inorganic acids markedly interrupted the normal course of the fermentation, especially when added at the rate of 0.5 gram per 100 milliliters calculated as lactic acid. In subsequent experiments artificial green olives were produced under laboratory conditions by addition of inorganic acids, but no appreciable fermentation took place and the characteristics of the fermented green pickle were lost.

Under ordinary conditions it is unnecessary to acidify the brines of green olives to control the fermentation. It is therefore recommended that acidification be considered only as a last resort to control the fermentation; 100-grain vinegar or 50 per cent edible lactic acid should be used.

MICROBIAL DECOMPOSITION

Bacteria, yeasts, and molds may cause deterioration of green olives during the pickling process, during subsequent storage of the fruit in barrels, or in the package finally used for distribution. As far as is known at present, bacteria are responsible for the most serious losses. The yeasts and molds have been found to cause trouble at times, particularly through lack of care on the part of the packer.

Bacteria often cause serious losses in spite of the care given to control of the fermentation and subsequent storage of the pickled fruit. Deterioration by yeasts and molds, on the other hand, generally reflects abject neglect during the final phases of fermentation and subsequent storage in barrels.

As already stressed, the course of biological, chemical, and physical events taking place during the primary stage of the fermentation determines in large measure the extent of deterioration of the fruit.

Gassy Deterioration.—The most common abnormality observed during the primary stage of fermentation is known as “floater,” “fish-eye,” or gas-pocket spoilage. The effects of such deterioration on the appearance and structure of the olives are shown in figure 18. This gassy de-

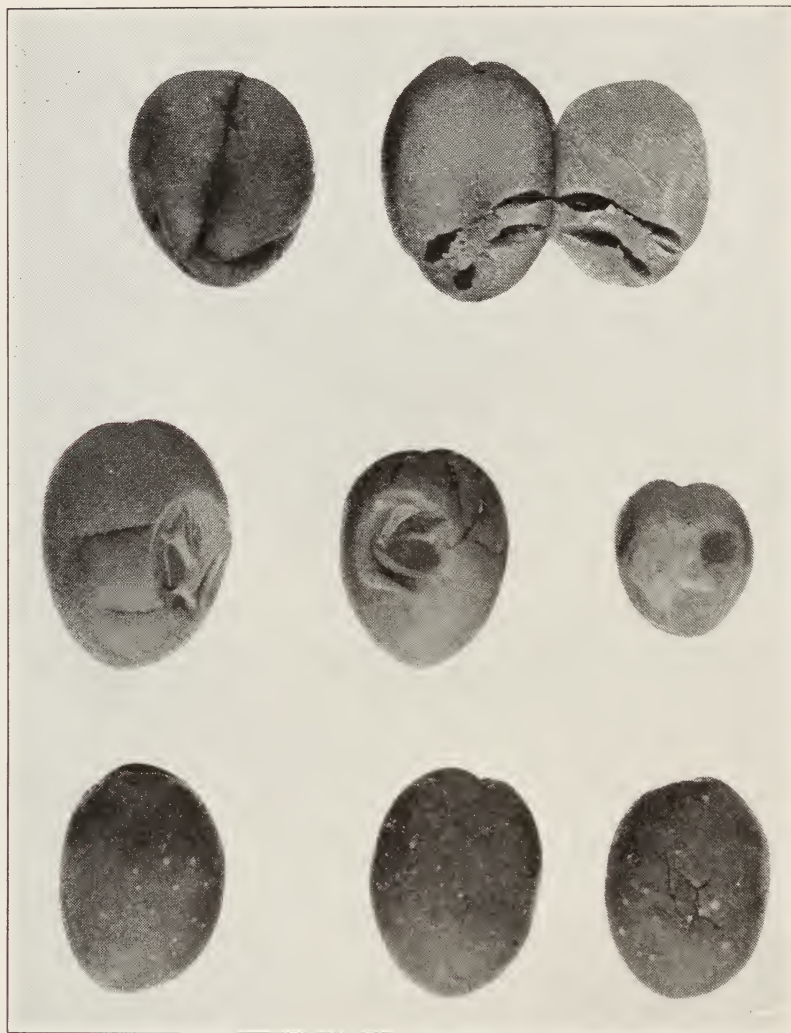


Fig. 18.—Microbial deterioration of green olives. Upper row: gas-pocket formation. Middle row: gas pockets accompanied by blister formation. Lower row: so-called "yeast spots." In reality most white spots on Spanish-type green olives are colonies of lactic acid bacteria which have developed just beneath the epidermis of the fruit.

terioration is characterized by the formation of blisters resulting from accumulation of gases which separate the skin from the flesh of the olives or by the formation of fissures or gas pockets (directly under the skin) which may extend to the pit of the fruit. As this type of spoilage is not confined to the green pickled olive alone, but occurs with all types of olives, it has been extensively studied by Cruess and Guthrie (1923),

Alvarez (1926), Tracy (1934), and West, Gililand, and Vaughn (1941). As a result of these investigations it is well established that the coliform bacteria are chiefly responsible for gassy deterioration.

The species most commonly encountered are *Aerobacter aerogenes* and *A. cloacae*. These two species accounted for 70.5 per cent of the coliform bacteria isolated and studied by West, Gililand, and Vaughn (1941). "Intermediate" bacteria (*Escherichia freundii* and *E. intermedium*) constituted the remaining 29.5 per cent. No true *E. coli* cultures were encountered.

Control of the coliform bacteria generally is accomplished satisfactorily during the primary stage of fermentation by the combined effects of salt brine, rapid increase in acidity, and microbial competition for the fermentable substances of the olives. However, in the case of abnormal fermentations where, for some reason, the desirable lactic acid bacteria are unable to dominate the microbial population, severe losses sometimes result from the activity of the coliform bacteria.

The most feasible control measures for commercial practice include the use of salt brines which stabilize at 7 to 8 per cent sodium chloride, steps being taken meanwhile to insure the immediate activity of the desirable lactic acid bacteria by the use of starters. Inoculation should be followed by addition of supplementary sugar only when laboratory examination has shown that the coliform bacteria constitute a minor portion of the microbial population or are absent from the brines.

In cases of prolonged, chronic spoilage where fruit is deteriorating slowly it is wise to attempt to control the microbial population with large inoculations of pure-culture starter or normal, actively fermenting brine.

In cases of acute spoilage, where there is potential danger of complete loss of the fruit, speedy rebrining with acidified brine is recommended to check the spoilage.

Many of the common species of *Aerobacter* encountered in spoiled olives can grow well in the presence of 7 per cent salt when the olive brine or culture medium is at about pH 7.0. However, as the pH of the brine or culture medium is decreased the effect of the salt is markedly increased.

Experimental results have shown that rather high resistance to salt may be acquired by cultures of *Aerobacter* allowed to remain in contact with 8 per cent salt for varying lengths of time. Several pure cultures of *Aerobacter* became sufficiently acclimatized to grow and produce gas in a glucose medium containing 12 per cent salt. A few cultures of *A. cloacae* grew in the presence of 14 per cent salt contained in the glucose medium, but did not produce gas when incubated at 30° C for 4 days.

Unfortunately these experiments had to be terminated before it was possible to determine the maximum salt concentration to which the *Aerobacter* cultures could be acclimatized. Nevertheless, this phenomenon is believed to be of common enough occurrence in the industry to warrant stressing once more that every effort must be made to establish the lactic acid fermentation as soon as possible after the olives are barreled and brined. Once the lactic acid bacteria dominate the fermentation, further trouble with the coliform bacteria is eliminated.

Butyric acid bacteria (the species *Clostridium butyricum*, and others) also cause gassy deterioration of olives. The butyric acid bacteria, however, are more important because they cause the malodorous butyric acid fermentation; consequently they will be discussed separately.

Species of the genus *Aerobacillus* also have been found to cause gassy deterioration of olives. These aerobacilli also cause softening of the olive flesh. Unfortunately, at present little is known of these bacteria as related to the deterioration of olives. Pure cultures have been isolated from olives, and softening and gas-pocket formation have been demonstrated under laboratory conditions.

Yeasts have also been reported to cause gassy deterioration of olives. Investigations with pure cultures of yeasts conducted recently have failed to substantiate this view with assurance. It is the belief of the writers that those bacteria which produce hydrogen gas as an end-product of the decomposition of organic compounds (such as glucose and mannitol) are the only microorganisms which are dangerous. Yeasts do not produce hydrogen as an end-product of fermentation although they do form large amounts of carbon dioxide; the same is true of the heterofermentative lactic acid bacteria (*Leuconostoc mesenteroides*, *Lactobacillus brevis*, and others).

Carbon dioxide is appreciably more soluble in liquids than is hydrogen gas. Furthermore, most if not all bacteria producing hydrogen gas are motile and therefore can invade the olive tissues much more readily than the immotile yeasts and lactic acid bacteria. If this supposition is not true why then is there not more gassy spoilage? Yeasts and lactic acid bacteria abound in every barrel of olives undergoing fermentation.

Malodorous Fermentations.—The butyric acid bacteria have been associated with the deterioration of olives for a good many years. This abnormal fermentation is characterized by its butyric acid or rancid aroma. In the early stages of the fermentation the odor is distinctly that of butyric acid or rancid butter; but, as the spoilage progresses, the odor becomes more pronounced and finally results in a very malodorous fecal stench.

Pure cultures of butyric acid bacteria have been isolated from a large

number of samples of spoiled green olives. Most of the cultures are closely related to or identical with *Clostridium butyricum*. All of the cultures isolated are sugar-decomposing types not capable of digesting protein. All of the isolates cause malodorous fermentation of olives if conditions (such as salt concentration, pH, and sugar content) in the brine are favorable for their growth.

Butyric acid spoilage is less widespread than the abnormal gassy fermentation caused by the coliform bacteria. Nevertheless, losses are extensive because *recovery of the olives affected by butyric acid fermentation is impracticable, unwise, and insalubrious*. Control therefore must be designed to prevent the activity of the butyric acid bacteria rather than to check the fermentation once it is started.

Control of the primary stage of fermentation is the most feasible industrial method for preventing the butyric acid fermentation. The recommendations made for control of the gassy fermentation are satisfactory for control of the butyric acid bacteria. It should be emphasized, however, that some of the cultures of *Clostridium butyricum* isolated from olives can grow in media having a pH of below 4.5; therefore, it is necessary to control the fermentation for a longer time than that necessary to successfully combat the coliform bacteria. Supplementary sugar (Cerelease) should be added in a quantity sufficient to insure acid production to proceed until the pH has decreased to 4.0.

Zapatera Spoilage.—Another malodorous fermentation of olives called *zapatera* by the Spaniards has caused deterioration of olives in California. This abnormal fermentation is characterized by the development of a very penetrating, unpleasant odor in green olives. The odor has been described as “sagey” by Ball (1938), Cruess (1941), and others. The odor of *zapatera* spoilage is relatively mild as compared with the rancid to malodorous stench caused by the butyric fermentation.

Zapatera spoilage was common in green olives in California during the 1935 to 1937 pickling seasons. It has become increasingly rare during the ensuing years, and now the recognition of true *zapatera* spoilage and its differentiation from the butyric fermentation have become greatly confused in the industry. At present the tendency exists to designate any abnormal odor emanating from olives as *zapatera*.

Under California conditions *zapatera* spoilage, unlike the butyric fermentation, occurs late in the final stage of fermentation when the reaction of the brine does not decrease below pH 4.5. At the time of onset of deterioration, although the total titratable acidity apparently may be fairly high, the pH of the affected brines increases as the spoiling progresses.

Smyth (1927) reported the results of a study of the bacteriology of

the Spanish green olive after having spent some time in Spain investigating the fermentation of such olives. Discussing the problem of spoilage, Smyth wrote :

Characteristic spoilage, termed *zapatera*, is detectable by taste, odor, and consistency, and is always associated with lowered acid values. While never endangering life or even health, this spoilage may result in losses of thousands of dollars to the packers. Spoilage is not due to any one organism but to one or more of a group of sporeforming, proteolytic, facultative rods normally present in the soils of Andalusia. These organisms develop well in brines up to and well over the salt content of olive brine, but are inhibited by lactic acid. Deterrination of pH values by means of brom-phenol blue is the best single check on the keeping quality of green olives. They should test to pH 3.8 or, better, pH 3.6 or 3.7.

Studies conducted thus far have failed to incriminate butyric acid bacteria as causative organisms of *zapatera* spoilage as known in California. On the basis of Smyth's report it seems reasonable that the *zapatera* and butyric acid abnormalities are caused by different microorganisms. Thus far, investigations conducted by the present writers have failed to detect the bacteria reportedly responsible for true *zapatera* deterioration, nor has it been possible to reproduce the spoilage in olives pickled in the laboratory.

Control of the fermentation of green olives in California so that the final pH of the brine is decreased to pH 4.0 or below has markedly reduced losses due to *zapatera* spoilage. In order to insure sufficient acid formation to decrease the pH to the safe level it is necessary in most instances to add supplementary sugar to the fermenting olives.

"*Yeast Spots.*"—One of the most common defects associated with green olives pickled in California is the abnormality known throughout the industry as "yeast spots." This defect of pickled green olives, shown in figure 18, is characterized by the formation of raised white spots or pimples between the inner surface of the epidermis and the flesh of the olive. These small white spots actually are colonies of bacteria or yeasts which have developed during the fermentation. Although commonly called "yeast spots," in reality most of the pimples examined in the laboratory have contained lactobacilli. When these bacteria have been isolated and studied, they have been identified as *Lactobacillus plantarum*. This defect is also found in green olives imported from Spain. Control is not known. Evidently, any factor which influences the entrance of the microorganisms through the pores or other breaks in the skin of the olive may contribute to the development of these colonies. Since such olives are considered unsightly some loss of value of the pickled fruit is experienced although the olives are perfectly normal and healthful in other respects.

Softening.—Softening of green olives during pickling occurs infrequently in California. Those cases which have been observed invariably have been found to result from improper control of the lactic acid fermentation. Under conditions where softening causes appreciable losses it commonly is found associated with the butyric fermentation. Laboratory examination has demonstrated that softening of the olive tissue results from destruction of the pectic substances which give the olives firmness and form.

The softening conceivably may result from the activity of bacteria, yeasts, or molds, for it is known that certain representatives of each of these three groups of microorganisms possess strong pectinolytic powers.

Cultures of the genus *Aerobacillus* which have been isolated from California green olives have been demonstrated to possess the ability to cause the softening of olive tissue. The problem has been investigated, but more work must be done before conclusions are warranted.

Control of softening, as with the other types of spoilage, is associated with control of the desirable lactic acid bacteria. A normal, active lactic acid fermentation is the best preventive measure. Once softening occurs, the olives lose their value and should be discarded.

Other Abnormalities.—Other defects of pickled green olives have been observed. For the most part, these defects are due to foreign flavors and aromas in the pickled fruit. One of the commonest defects observed has been the whisky flavor imparted to olives as a result of pickling the fruit in improperly cleaned barrels which were first used for storing and aging whisky.

Heavy yeast and mold growths on the surface of brines in improperly filled barrels of olives also impart off-flavors and aromas to the fruit during fermentation and subsequent storage. Aside from the off-flavors and aromas developed by such neglect, it is known that the yeasts and molds rapidly destroy the lactic acid contained in the brines (Cruess, 1930, and many other investigators). Loss of lactic acid in this manner predisposes the olives to further decomposition by bacteria. Control of yeast and mold growth is best accomplished by routine rebrining of the barrels with fresh brine, and keeping them tightly closed during storage to eliminate air.

Sediments and Gas Formation in Packaged Green Olives.—Unightly sediments and gas formation frequently occur in glass-packed green olives. Such deterioration is to be avoided, for the consumer has been educated to demand green olives packed in bright, clear brine.

One of the chief causes of sediments and gas formation in packaged green olives is the presence of fermentable material that has not been decomposed. If such material remains in the olives at time of packing

in glass, heavy sediments and gas formation in the brines appear quickly unless other preventive measures are taken.

The lactic acid bacteria should be removed by either washing or pasteurizing, for they can gain energy for growth and multiplication from lactic acid, albeit slowly.

Most sediments are composed of lactic acid bacteria; but if the containers are not completely filled or well sealed, yeasts may form much of the sediment.

Control of clouding and sediment formation in the brines of glass-packed olives includes the following measures:

1. Determination that all fermentable material in the olive has been decomposed.

2. Thorough washing of the pickled olives to remove lactic acid bacteria adhering to the surface of the fruit.

3. Use of fresh brine made with high-quality salt and edible lactic acid.

4. Complete filling of the jars with brine to exclude air. (Vacuum sealing or pasteurization will have the same effect if properly executed.)

5. Pasteurization at 130° to 140° F to destroy yeasts and bacteria.

Ayers and associates (1928), Mullen (1929), and Cruess (1930) have investigated the problem of sediments and other defects in packaged olives.

PACKAGING AND SHIPPING OF PICKLED GREEN OLIVES

When the green olives have undergone complete fermentation and have "cured," acquiring the well-known green Spanish-olive color, flavor, and aroma, the fruit is then ready for sale. The majority of the California pack at the present time is shipped to repackers in all sections of the United States. Only a small portion of the pickled olives are packaged in glass in California. The proportion of green olives so packed in this state, as compared with the total national pack, is inferred from table 13 where the value in dollars of the California pack, as compared with the total United States pack, is shown.

At time of preparation of the pickled fruit for glass-packing locally or for shipment to repacking centers throughout the nation, the following conditions should have been attained:

1. The fermented olives should have developed from 0.6 up to 1.0 or 1.25 grams of total acidity expressed as lactic acid per 100 milliliters of brine.

2. The pH of the brine should have decreased to between about pH 4.3 and pH 3.8 or 3.9.

3. The flesh of the fruit should have lost its raw taste and flavor.

4. The raw green color of the unpickled, lye-treated, and washed fruit must have changed to the golden olive-green shade characteristic of the Spanish olive.

5. The pickled olives must have cured to the desirable flavor and aroma characteristic of the Spanish olive.

The pickled fruit is removed from the barrels to be sorted for color and quality, and is graded for size if originally barreled and fermented

TABLE 13
THE VALUE OF SPANISH-TYPE GREEN OLIVES PACKED
IN GLASS IN CALIFORNIA, COMPARED WITH THE
TOTAL PACK FOR THE UNITED STATES

Year	Value in dollars	
	California	United States total
1925		5,752,770
1927		3,592,385
1929	10,611	8,244,733
1931	195,758	6,809,299
1935	461,991	4,333,230
1937	398,841	4,183,335
1939	381,887	6,127,410

Source of data: Western Canner and Packer 34 (7):27-28. 1942.

as "orchard run." These quality grades are similar to those used by the importers for grading Spanish green olives.

Sorting and grading for quality are done by hand on sorting belts similar to those used for the fresh green fruit. The high acidity, plus the salt content, makes the brines from the pickled fruit very corrosive; consequently some of the packers have installed continuous belts and stationary table tops of stainless steel in the sorting and grading rooms used exclusively for handling pickled green olives.

The olives sold for shipment to the various repacking centers are graded for quality and size and then rebarreled in 50-gallon oak barrels. Some packers rebrine the graded fruit with fresh salt brine (about 28° to 30° salometer), which may or may not be acidified with edible lactic acid. Others use the original brine plus a small amount of new clear salt brine for brining the graded fruit. There is no uniformity in this respect, for the olives are prepared for shipment to conform with the specifications of the orders received.

Table 14 shows the range of acidity and salt content of the brines of various samples of glass-packed green olives fermented in Spain and in California, together with other data on packs for retail trade. More

TABLE 14

COMPARISON OF RETAIL PACKS OF CALIFORNIA SPANISH-TYPE GREEN OLIVES WITH THOSE IMPORTED FROM SPAIN

Packer	Variety	Size grade	Type of pack	Declared weight of olives, ounces	Vacuum, inches	Lactic acid, grams per 100 milliliters brine	pH of brine	Salt, grams per 100 milliliters brine	Condition of brine	Remarks
Olives known to be pickled and packed in California										
A.....	Manzanillo	Medium	Pitted	6½	2	0.450	3.53	7.25	Clear; no sediment	No defects of fruit; best sample of all
B.....	Sevillano	Giant	Whole	11	0	.651	3.46	7.02	Clear; no sediment	Color not uniform; "yeast spots"; taste unsavory
C*.....	Sevillano	Larger than Super Colossal	Whole		0	.441	3.66	7.02	Clear; no sediment	Extra fancy fruit; best Sevillano sample
D*.....	Sevillano	Super Colossal	Whole		0	.729	3.40	7.25	Clear; no sediment	Color fair; "yeast spots"; too sour
E.....	Manzanillo	Medium	Whole	11	0	.459	3.60	6.32	Clear; no sediment	Color too brownish; a few "yeast spots"; insipid
	Sevillano	Colossal	Whole	6½	0	.522	3.54	7.07	Clear; no sediment	Color good; "yeast spots"; average in taste
	Sevillano	Whole fruit; Colossal; stuffed fruit; Mammoth Large	Whole and pimento-stuffed	6½	0	.522	4.00	7.49	Clear; no sediment	Color good; "yeast spots"; average in taste
F.....	Manzanillo	Pimento-and almond-stuffed	Pimento-and almond-stuffed	4	0	.378	3.75	7.02	Clear; no sediment	Color even but light; average in taste
	Manzanillo	Medium	Whole	2	0	.423	3.60	6.90	Clear; no sediment	Color even but light; too much wood taste, not oak
	Manzanillo	Small	Pimento-stuffed	4	0	.324	3.60	7.55	Clear; no sediment	Color good; average in taste; pimento color too uneven
	Manzanillo	Small	Pimento-stuffed	1½	0	.333	3.42	7.08	Clear; no sediment	Color good; ragged stuffing; average in taste
G.....	Manzanillo	Small	Whole	3¼	0	.513	3.49	8.25	Clear; no sediment	Color very poor; discolored flesh and skin; average in taste

H.....	Manzanillo	Medium	Pimento-stuffed Whole	1 $\frac{3}{4}$	0	.504	3.44	8.66	Clear; no sediment	Color good; ragged stuffing; superior flavor and aroma
I.....	{Manzanillo	Medium		2	0	.468	3.41	7.79	Clear; no sediment	Satisfactory color; objectionable medicinal taste
	{Manzanillo	Small	Pimento-stuffed	1 $\frac{1}{2}$	0	.504	3.41	7.31	Clear; no sediment	Satisfactory color; objectionable medicinal taste

Olives purported to have been pickled in Spain and packed in the United States

J..... (New York)	{Manzanillo?	Substandard (very small) Large	Pimento-stuffed Whole	1 $\frac{1}{2}$	0	.639	3.35	7.20	Clear; no sediment	Color good; "yeast spots"; woody, inferior texture; average in taste
K..... (Chicago)	{Manzanillo	Jumbo	Whole	6	0	.504	3.32	7.49	Clear; no sediment	Color good; "yeast spots"; average in taste
	{Manzanillo	Large	Celery-stuffed	2	0	.405	3.35	7.25	Clear; no sediment	Color even but light; "yeast spots"; savory
L..... (San Francisco)	{Manzanillo	Medium	Pimento-stuffed	3	0	.387	3.94	6.26	Clear; no sediment	Unnatural green color; indications of lye-peeling
	{Manzanillo	Medium	Pimento-, and onion-, and almond-stuffed	3	0	.630	3.47	8.31	Clear; no sediment	Color uneven; average in taste
	{Manzanillo	Medium	Pitted	3	0	.630	3.45	6.26	Clear; no sediment	Color good; onions too soft; otherwise satisfactory
M..... (Chicago)	{Manzanillo	Medium	Whole	2	0	.297	3.34	6.97	Clear; no sediment	Color a bit light; texture good; insipid
	{Manzanillo	Large	Whole	1 $\frac{3}{4}$	1 $\frac{1}{2}$	.558	3.40	7.25	Clear; no sediment	Color good; "yeast spots"; woody, granular texture
	{Manzanillo	Small	Pimento-stuffed	2	1	.648	3.36	6.32	Clear; no sediment	Color good; pimento too soft; average in taste
N..... (San Francisco)	{Manzanillo	Giant	Whole	3 $\frac{1}{2}$	0	.842	3.55	6.44	Cloudy; no sediment	Color good; no defects; savory flavor and aroma
	{Manzanillo	Small	Pimento-stuffed	2	0	.261	3.37	6.97	Clear; no sediment	Color good; slightly insipid
O..... (San Francisco)	{Manzanillo	Substandard (very small)	Pimento-stuffed	1 $\frac{1}{4}$	0	0.360	3.89	7.84	Clear; no sediment	Color good; texture of olives and pimento too soft

* Samples from gallon containers repacked in the laboratory with original brine.

TABLE 14 (Concluded)

Packer	Variety	Size grade	Type of pack	Declared drained weight of olives, ounces	Vacuum, inches	Lactic acid, grams per 100 milliliters brine	pH of brine	Salt, grams per 100 milliliters brine	Condition of brine	Remarks
Olives purported to have been pickled in Spain and packed in the United States										
P. (New York)	Sevillano	Large	Whole	2¼	Cork seal	0 432	4 22	8 54	Cloudy; no sediment	Color good; texture woody and granular; musty, corky flavor
Q. (Salt Lake City)	Sevillano	Jumbo	Whole	Bulk	Bulk	369	4 05	7 25	Original brine	Color fair; "yeast spots"; shriveled; too much wood taste, not oak
R. (Chicago)	Sevillano	Large	Whole	1¾	3 5	180	3 56	9 19	Cloudy; no sediment	Color, gray; interior flesh, gray; unsavory, insipid
S. (Salt Lake City)	Sevillano	Giant	Whole	6½	0	815	3 60	7 61	Cloudy; slight sediment	Color good; "yeast spots"; average in taste
T. (St. Louis)	Sevillano	Whole fruit; Colossal; stuffed fruit; Mammoth Medium	Whole and pimento-stuffed	6	0	459	3 50	8 66	Clear; no sediment	Color uneven; "yeast spots"; insect injury; average in taste
	Manzanillo	Medium	Pimento-stuffed	3	0	572	3 10	7 02	Clear; no sediment	Color good; stuffing not all Spanish sweet pepper as declared, but a paste material
U. (Omaha)	Sevillano and Manzanillo	Extra large	Whole and pimento-stuffed	5	0	0 297	3 70	6 91	Slightly cloudy; no sediment	"Yeast spots" on whole and stuffed fruit. Stuffing made by packing pit cavity with material which might be partly pimento, and capping with circle of pimento skin

attention to closer standardization of acidity and salt content of the brines of packaged green olives is clearly desirable on the part of producers here.

As already stated, the two commonest defects of green, fermented olives are poor color and the presence of "yeast spots." This is equally true of olives pickled in California or Spain, on the basis of the examination of retail samples shown in table 14.

One point of interest that should receive particular attention of picklers and packers is shown in the relation of total acidity to pH. The samples most insipid to taste had a satisfactory pH level (below 4.0) but also had a very low total acidity expressed as grams lactic acid per 100 milliliters. It is very evident that determination of pH value alone cannot be used as an index for quality or taste of the finished product.

The two best samples examined in this particular experiment were pickled and packed in California under strict laboratory supervision at all times. In general, the quality of the fruit regardless of origin was satisfactory but not superior; the quality of the California green olives was as good and as bad as that of olives supposedly pickled in Spain, as can be seen by examination of table 14.

All samples used for the study were obtained from retail stores with the exception of two 1-gallon samples which are designated in the table. Although the sampling is not large enough to warrant statistical treatment, it is felt that the olives examined are representative of the different packs and qualities of California and Spain offered for sale to the average retail customer.

The olives to be packed in glass at the original packing plant or in a repacking center are first checked carefully to see that sorting and grading were accurate. The olives are then packed into various sizes and shapes of glass containers.

If the olives are to be packed according to a definite pattern, each olive must be carefully placed in the jar with wooden or noncorrosive metal tongs or other instrument. "Pattern" packaging requires dexterous hand labor and is commonly used for the highest-quality fancy, whole, or pitted and stuffed fruit. For an ordinary pack the olives are filled into the containers at random.

After packing the olives in glass the containers are filled with water or washed in some other fashion, and inverted to drain in order to remove all traces of sediment which adhere to the fruit. It is important to remove as much of the sediment as possible. After the washed olives have drained properly, the jars are completely filled with a fresh brine of about 28° to 30° salometer, and sealed. Sometimes the brine is acidified with 0.25 to 0.50 grams edible lactic acid per 100 milliliters of brine.

The packed and sealed olives usually are not sterilized or pasteurized, but later sedimentation is decreased if they are pasteurized at about 140° F.

The original brine from the fermented olives is sometimes used; but such brine, although retaining much of its original and desirable flavor and aroma, predisposes the packaged fruit to unsightly sediment caused by the growth of bacteria or yeasts in the bottles or jars of fruit. Original brine must be clarified and filtered; the retail trade has been educated to expect and demand green olives packed in clear, water-white brine.

Formerly most of the pitted green olives both plain and stuffed, were imported from Spain, where the fruit had been prepared and packed in barrels prior to shipment to the United States for repacking in glass containers.

The development of the pitted, canned ripe olive and increased production of the Spanish-type green olive in California have been responsible for the development of ingenious pitting machines which far surpass the hand-operated pitting machines used in Spain. These machines are used for pitting either green or ripe olives of various sizes and varieties.

The pitted green olives are commonly stuffed with small strips or “plugs” of brine-stock pimento which are folded and stuffed into the olive by hand. Canned pimentos are used only when a supply of brined pimentos is not available; but the latter are preferred because of the crisper, firmer texture as well as the lower cost.

The pitted and stuffed olives are then packed in barrels, which are filled with original or fresh brine and allowed to ferment further; all of the fermentable material of the pimento stuffing must be decomposed to avoid gas formation and unsightly microbial clouding and sedimentation in the glass-packed fruit.

Pitted olives are filled with a wide variety of stuffings other than pimento; some of these are pearl onions, pickled celery, almonds, and anchovies. Pitted olives, sold without stuffing, afford the opportunity to serve with many unusual fresh vegetable and cheese or meat “plugs” which cannot be kept for any length of time or which lose their distinctive characteristics when preserved in the acidified, salty brine used for green olives.

LABORATORY CONTROL

The necessity for laboratory analyses for adequate control of the production of green fermented olives has been shown repeatedly in the foregoing discussion. As already stressed, the quality of the finished product is dependent upon thorough technical control of the whole process.

As with other food industries, the scientific and practical aspects of green-olive pickling are so closely related that training in and practical understanding of bacteriology and chemistry should be considered mandatory requirements for the laboratory personnel; from the utilitarian point of view, constant laboratory control is essential to avoid unnecessary losses.

The most important technical control measures for which the laboratory should be responsible include :

1. Accurate control of the concentration of sodium hydroxide used for treating the olives to destroy the bitter glucoside.

2. Accurate control of the concentration of the salt used in the brines.

3. Determination of acid production in the brines of the fermenting olives by noting the progress of increase in titratable acidity and decrease in pH values.

4. Determination of fermentable compounds (sugar and mannitol).

5. Recognition of the various desirable bacteria and detection of microorganisms causing spoilage.

In addition, the laboratory personnel should understand and be able to employ measures to combat spoilage; should be responsible for supervision of the care of the barreled fruit during fermentation and subsequent storage of the pickled fruit; should collect processing and production data for all fruit pickled, and collect and store representative samples of all pickled fruit sold; and, in coöperation with the operational personnel (plant superintendent, etc.), should plan production schedules and estimate materials needed for each pack of green, fermented olives.

Proper lye treatment of the fresh, green olives is important enough to warrant accurate estimation of the sodium hydroxide content of all lye solutions used. Hydrometry has been used commonly for this determination. Since the hydrometer is accurate only when pure compounds are dissolved in pure water, it is readily seen that the hydrometer method may be somewhat inaccurate. Under conditions existing in the industry, hydrometry is not to be recommended unless the value of accurately standardized conditions is recognized and trained personnel is in charge.

Determination of Total Alkalinity.—If the commercial lye used contains 95 per cent or more of sodium hydroxide and the water supply is not high in carbonate content, titration of total alkalinity is a sufficiently accurate estimation for practical application.

For the determination of total alkalinity, pipette 10 milliliters of a representative sample of the stock lye solution into a 250-milliliter beaker or flask containing 100 to 150 milliliters of distilled water and

3 to 5 drops of methyl orange indicator. Titrate with 0.25*N* HCl until the orange color just turns to a reddish color. The total alkalinity, expressed as grams of sodium hydroxide per 100 milliliters, is obtained by multiplying the number of milliliters of 0.25*N* HCl used in titrating by 0.10 (1 milliliter of 0.25*N* HCl is equivalent to 0.01 gram of NaOH).

Determination of Sodium Hydroxide in the Presence of Carbonates.—If a more accurate analysis of the sodium hydroxide content of the lye solution is required, then the determination must take into account the carbonate content of the lye and water used in preparation of the solution.

To determine the amount of sodium hydroxide contaminated with carbonates, pipette 10 milliliters of a representative sample of the stock solution into a 250-milliliter beaker. Add 100 milliliters of distilled water, 2 or 3 milliliters of 20 per cent barium chloride solution, and 3 to 5 drops of phenolphthalein. Stir well to allow the barium chloride to react with the carbonates to form insoluble barium carbonate. Titrate with 0.25*N* HCl until the red-colored solution just turns colorless. *Titration must be accompanied by constant stirring to prevent reaction of the acid with the barium carbonate.* The sodium hydroxide content, expressed as grams of NaOH per 100 milliliters, is obtained by multiplying the number of milliliters of 0.25*N* HCl used in titrating by 0.10.

Determination of Sodium Chloride Content.—Although hydrometers (salometer, salinometer, or Baumé) are commonly used for estimating the sodium chloride content of the brines used for pickling green olives, such determinations may be very inaccurate; the criticisms valid for the estimation of sodium hydroxide content of the lye solutions apply even more strikingly to the estimation of sodium chloride content of brines by hydrometry.

Volumetric estimation is to be recommended and the following procedure is satisfactory for determining the sodium chloride content of brines used for pickling, storing, and packaging green, fermented olives.

One milliliter of a representative sample of the brine to be analyzed is added to 200 milliliters of distilled water containing 1 milliliter of 0.5 per cent potassium chromate solution. The sample is then titrated with 0.1*N* silver nitrate solution until a slight, permanent red color appears. The sodium chloride content, expressed as grams of NaCl per 100 milliliters of brine, is obtained by multiplying the number of milliliters of 0.1*N* AgNO₃ solution used in titrating by 0.585.

Determination of Total Acidity.—A steady increase in acidity of the brine during fermentation of green olives is a measure of the activity of the desirable lactic acid bacteria. Ten milliliters of a representative sample of brine is added to 250 milliliters of boiling, distilled water

contained in a 500-milliliter Erlenmeyer flask. Three to five drops of phenolphthalein are added and the solution is titrated with 0.1N NaOH until a faint pink color appears. Green olive brines generally change to a darker color just before the phenolphthalein endpoint is reached. Disregard this first change in color and titrate until a faint pink color appears. Total acidity, expressed as grams of lactic acid per 100 milliliters of brine, is obtained by multiplying the number of milliliters of 0.1N NaOH used in titrating by 0.09 (1 milliliter of 0.1N NaOH is equivalent to 0.009 gram lactic acid). By adjusting the normality of the NaOH solution to 0.111N the grams of lactic acid per 100 milliliters of brine can be read directly from the burette. The burette reading multiplied by 0.1 gives the correct value.

Determination of pH Values.—It is not the purpose of this discussion to delineate the background necessary for a thorough understanding of the measurement of hydrogen-ion concentration and determination of pH. Those interested should consult such authorities as Clark (1928), La Motte, Kenny, and Reed (1932), Kolthoff (1937), Kolthoff and Laitinen (1941), Dole (1941), or other texts and original literature.

The pH value is an expression of the hydrogen-ion activity or concentration of hydrogen ions in a solution. Hydrogen ions are responsible for the sour taste of acids. The soapy feel and brackish taste of dilute lye solutions result from the presence of hydroxyl ions.

The pH scale is a simplified means of denoting the intensity of acidity and alkalinity in terms of hydrogen-ion concentration just as the centigrade or Fahrenheit scales are measures of the intensity of heat and cold.

Hydrogen-ion concentration may be expressed in several different ways. In terms of grams per liter, pure water contains 0.0000001 gram of hydrogen ions; in powers of 10 this figure becomes 1×10^{-7} ; the reciprocal of this concentration of hydrogen ion is 10,000,000 or 10^7 ; the logarithm of the reciprocal of the weight of hydrogen ions in grams per liter is 7.0. This logarithm is called the pH value. The pH values range from 0.0 (strongest acidity) to pH 14.0 (strongest alkalinity). The neutral point is pH 7.0; decreasing numbers below 7 denote increase in acidity; increasing numbers above 7 denote increase in alkalinity.

Two methods are available for determining pH values: the colorimetric method using acid-base indicators, and the electrometric method using potentiometric instruments with one of several types of electrodes.

Colorimetric determination of pH is based on the fact that various indicators change in color when acted upon by acid or alkaline solutions. The "spot-plate" and "block-comparator" methods are the two techniques commonly used.

Potentiometric determination of the pH of olive brines at the present time is made largely with several types of industrial-model instruments employing the glass electrode. The various chemical and apparatus companies supply either type of apparatus and chemicals needed for operation; and directions for operation accompany the equipment.

It is to be stressed again that pH control of the brines of green olives during fermentation, storage, and packaging is essential; and trained personnel thoroughly familiar with the limitations of either method of determining pH should be responsible for such control.

Estimation of Reducing Sugars.—The necessity for detection of the presence or absence of fermentable material (simple hexose sugar and mannitol) in green-olive brines has been stressed repeatedly. For practical purposes, in routine control during fermentation and storage prior to packing in glass, qualitative determination of sugar is sufficient. The qualitative test is made by use of the well-known copper reduction method.

Immediately before making the test, prepare the Soxhlet reagent by mixing equal portions of modified Fehling's A and B solutions into a clean, dry flask. Pipette 10 milliliters of the mixed Soxhlet reagent into a clean, dry test tube and then pipette 1 to 5 milliliters of the desired brine into the reagent mixture. Heat to boiling slowly and carefully to avoid bumping and sudden boiling, which might result in loss of the sample. The presence of reducing materials causes the reduction of the cupric hydroxide, $\text{Cu}(\text{OH})_2$, to cuprous oxide, Cu_2O . The blue color of the Soxhlet reagent is changed to a yellow to red color, according to the amount of reducing material present in the brine, and other factors.

Modified Fehling's A solution is prepared by dissolving 34.639 grams of copper sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, in water and making to a volume of 500 milliliters. Modified Fehling's B solution is prepared by dissolving 173 grams of sodium potassium tartrate (Rochelle salts) and 50 grams of sodium hydroxide in water and making to a volume of 500 milliliters.

The mixed solutions should be tested by boiling to determine whether the solution will of itself cause the formation of a precipitate of cuprous oxide.

In the many olive fermentations which have been tested using this method the substances other than simple hexose (glucose) and mannitol capable of reducing copper in alkaline tartrate solution apparently were absent; clearcut negative tests were always obtained at the time microbial and chemical changes indicated "sugar" was lacking.

This method is recommended since, for control purposes, the laboratory personnel is interested in knowing when all of the sugar has been utilized, either before adding more sugar to be converted to lactic acid

by fermentation or before glass-packing the pickled olives after fermentation and subsequent storage.

Recognition and Detection of Spoilage Bacteria.—The necessity for control of the spoilage bacteria and methods for their control have already been discussed. The recognition of the types of bacteria which are capable of spoiling green olives during the fermentation process is equally important.

The coliform bacteria, particularly *Aerobacter* species (*A. aerogenes* and *A. cloacae*), have been found to cause the most damage; the butyric acid bacteria (*Clostridium butyricum* and closely related species) are second in importance as far as is known at the present time.

Fortunately, the majority of the bacteria comprising these two groups are motile. Thus recognition of the important spoilage bacteria under control conditions is simplified. Routine microscopical examination of appropriate "hanging drop" slide preparations of samples from suspected brines for motile bacteria is sufficient for control purposes. Recognition of the presence of spoilage bacteria is materially aided by close observation of the fermenting olives for evidence of deterioration (such as gas pockets, off-odors, and retarded acid production).

Detection of the type of spoilage is simple, once deterioration of the fruit is evident. The olives shown in figure 18 are evidence of the destructiveness of the coliform bacteria. The characteristic rancid off-odor of butyric acid is considered sufficient evidence of the presence of the anaerobic butyric acid bacteria (*Clostridium butyricum* and other saccharolytic species).

To detect the presence of the different spoilage bacteria before deterioration is manifest, bacteriological analyses must be used.

Presumptive evidence for the presence of coliform bacteria is obtained by use of lactose broth inoculated with a representative sample of suspected brine and incubated at 30° C under aerobic conditions. Gas production in 24 to 48 hours may indicate the presence of coliform bacteria. Detailed methods are outlined in standard methods promulgated by the American Public Health Association (1936); in Levine (1933); or in other laboratory handbooks.

Presumptive evidence of the presence of butyric anaerobes is obtained by heating a sample of brine (neutralized with an excess of calcium carbonate) in boiling water for 10 to 20 minutes and then inoculating a suitable glucose broth or agar medium with 1 portion of the heated brine. The inoculated medium is then incubated at 30° C under anaerobic conditions. Gas formation in the glucose medium may indicate the presence of anaerobic sporeforming bacteria of the saccharolytic group.

Presumptive evidence for the presence of the desirable lactic acid

bacteria can be obtained by inoculating filtered tomato juice adjusted to pH 4.0 with a sample of brine and incubating the inoculated tomato juice at 30° C under anaerobic conditions. Lactic acid bacteria will cause clouding of the tomato juice. Anaerobic conditions prevent the growth of yeasts. Although anaerobic, sporeforming bacteria are capable of growing in tomato juice they have never been troublesome in this test.

For routine control, the presumptive tests are usually sufficient; methods of confirming the results are too detailed to discuss here. It is obvious that such control work should not be placed in the hands of untrained personnel.

TABLE 15
SOME OF THE CHEMICAL CONSTITUENTS OF PICKLED GREEN OLIVES
AS REPORTED BY VARIOUS INVESTIGATORS

Sample	Refuse (pits)	Chemical composition of the flesh							
		Water	Protein	Oil	Ash	Total carbo- hydrates	Fiber	Calories per 100 grams	Calories per pound
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>percent</i>	<i>per cent</i>	<i>per cent</i>		
Whole fruit (Atwater and Bryant, 1906).....	27	58	1 1	27 6	1 7	11 6	...	...	...
Whole fruit (Stathopoulos, 1925).....	22.5	67 96	1 56	15 26	10 20	5 02	1 05	...	...
Flesh (Chatfield and Adams, 1940).....	0	75 2	1 5	13 5	5 8	4 0	1 2	144	650
Whole fruit (Chatfield and Adams, 1940)....	21	59 4	1 2	10 7	4 6	3 2	0 9	133	515

CHEMICAL COMPOSITION OF GREEN, FERMENTED OLIVES

Unfortunately, adequate analyses of the chemical composition of green, fermented olives of the Spanish type are not available; such as exist represent only a few samples. Each investigator has been interested in some particular chemical component (oil, sugars, vitamins, etc.). With few exceptions, these analyses are of doubtful value because variations in constituents resulting from different varieties of fruit have not been recognized or recorded. The data shown in table 15 represent some of the analyses which have been published; the variety of olives sampled was not designated.

Oil Content.—As noted in table 15 the oil (fat) is the chief chemical constituent other than water which has apparent value as a food. The oil content of pickled green olives is known to vary according to the variety used for pickling. Typical analyses of the oil content of pickled

green Sevillano and Manzanillo olives are shown in table 16. The samples represent olives pickled in Spain and California. The analyses were made by Pitman (1930).

TABLE 16
COMPARISON OF OIL CONTENT, AND OTHER CONSTITUENTS OF THE FLESH OF
CALIFORNIA AND IMPORTED PICKLED GREEN OLIVES*

Sample no.	Variety	Oil content, per cent	Water, per cent	Lactic acid, per cent	Degrees salometer
California samples					
1	Manzanillo.....	16.06	75.0	0.396	23
2	Manzanillo.....	16.10	73.0	.180	29
3	Manzanillo.....	15.75	72.5	.207	28
4	Manzanillo.....	14.52	75.1	.225	27
5	Manzanillo.....	18.00	72.5	.189	28
6	Sevillano.....	12.85	73.2	.252	27
7	Sevillano.....	6.57	82.4	.279	27
8	Sevillano.....	7.19	81.1	.252	28
9	Sevillano.....	8.33	81.8	.297	27
10	Sevillano.....	11.48	76.3	0.513	38
	Average for Manzanillo.....	16.08	73.6	0.240	27
	Average for Sevillano.....	9.28	79.0	0.319	29
Samples from Spain					
1	Manzanillo.....	14.30	72.6	0.108	37
2	Manzanillo.....	17.50	72.9	0.170	32
3	Manzanillo.....	11.05	76.5	0.378	39
4	Sevillano.....	14.75	72.6	0.990	39
5	Sevillano.....	8.56	76.5	0.567	34
6	Sevillano.....	12.85	74.8	0.657	32
7	Sevillano.....	9.44	72.8	1.160	31
8	Sevillano.....	14.15	73.9	0.585	33
9	Sevillano.....	11.54	76.5	0.369	38
10	Sevillano.....	10.84	77.5	0.845	31
	Average for Manzanillo.....	14.28	74.0	0.219	36
	Average for Sevillano.....	11.73	74.9	0.739	34

* Data from Pitman (1930).

Vitamin Content.—It has been known for some time that pickled green olives contain the fat-soluble vitamin A. Booher and Marsh (1941) have analyzed Spanish-pickled green olives (bottled in New York) by the rat-growth method. The edible portion of the pickled fruit was found to contain about 1,000 International Units of vitamin A per 100 grams of flesh (edible portion). The variety of fruit was not designated.

Earlier analyses have shown the presence of varying amounts of vitamin A. These olives apparently contain insignificant quantities of the vitamin-B complex and vitamin C. The content of vitamin A is not high enough to be of particular significance.

SUMMARY OF RECOMMENDATIONS FOR IMPROVEMENT OF THE INDUSTRY

The investigations reported herein have led to several conclusions concerning the technical aspects of green-olive pickling:

1. The pickling must be done under constant laboratory supervision if the highest-quality pickles are to be obtained and if severe economic losses are to be avoided.

2. The course of biological and chemical events occurring during the primary stage of fermentation determines in large measure the success or failure of the subsequent phases of fermentation.

3. The chief problems which need further study involve investigation of the factors affecting attainment of satisfactory color; the direction and control of fermentation by use of starters; and the control of "yeast spots" and other deteriorations caused by microorganisms.

The value of constant laboratory supervision, the known factors involved in control of color, the use of starters, and the control of microbial deteriorations have already been discussed; but control of the primary stage of fermentation is so important that it merits further consideration here.

The gross microbial and chemical changes follow a definite sequence in normal fermentations of green olives. Known factors which influence the primary stage of fermentation and have an important bearing on the subsequent phases of fermentation include the fermentability of the olives; the strength of the salt brine; the total microbial population of the brine at the beginning of the fermentation; and the ratio of the desirable lactic acid bacteria to other bacteria and yeasts which constitute the microbial population of the salt brine used to cover the olives. The influence of most of these factors on fermentation has already been discussed. Additional research must be designed to investigate in detail the types of bacteria and yeasts which make up the microbial population during the primary phase of fermentation. Such investigation must be so conducted that the phenomenon of association and competition is thoroughly studied and properly understood.

Yeasts are invariably present, sometimes even predominant, during the primary phase of fermentation. Under certain conditions yeasts become so active during the primary phase of fermentation that they crowd out the lactic acid bacteria. What role do yeasts play in the normal fermentation of olives? Are the species the same in all fermentations? Is their presence desirable or should the yeasts be avoided if possible?

Similar questions relative to the role of certain bacteria found during the primary phase of fermentation must also be answered.

Control of the fermentation of olives is essential. This is accomplished by judicious use of starters of pure lactic acid bacteria or normal, actively fermenting brine supplemented by addition of sugar (Cerelese) when necessary. The use of starters has little effect on the ultimate flavor and aroma of the pickles but is necessary to insure development of the lactic acid fermentation and control of harmful organisms.

Poor color of the pickled green olives and the so-called "yeast spots" are the two most common defects, regardless of the place of origin. Poor color may be remedied by careful selection of the fresh fruit, proper lye-treatment and not too prolonged washing to remove the lye, followed by immediate barreling and brining of the lye-treated fruit. Barrels must be full of brine at all times, since olives exposed to the air darken rapidly.

No readily applicable preventive measure for the "yeast spots" is apparent, for these are generally colonies of true lactic acid bacteria (much less frequently yeasts) which grow between the epidermis and flesh of the fruit. These spots do not actually cause a deterioration of the olive tissue and have no sanitary significance. Even very select fruit may have such colonies. At present, efforts to standardize the glass-packed fruit with respect to total acidity, pH value, salt content, and creditable stuffing are much more important and merit more attention from the whole industry than the "yeast spots."

CONDENSED DIRECTIONS FOR PICKLING

The following briefly outlines the important steps involved in the production of green pickled olives:

1. The olives are picked at full size but of green to straw-yellow color. Care must be taken to avoid bruising, for all such marks are accentuated in the pickled fruit. Similar care must be taken in transporting the fruit to the factory and in subsequent handling of the fresh olives.

2. Transport the olives to the factory as soon as possible after picking. Long hauls of the fresh fruit are to be avoided.

3. Sort the fresh fruit for color and defects; size-grade or prepare to pickle the fruit "orchard run." The fruit should always be handled as soon as possible and be given adequate care.

4. Place the sorted and graded fruit at once in 1.5 to 2.0 per cent soda-lye solution. Sevillano olives frequently blister and peel when treated with too strong lye solution, and should be treated in not stronger than a 1.5 or 1.6 per cent solution.

5. Allow the lye to penetrate, on the average, about three fourths of the way to the pit of the olives.

6. Remove the lye solution and quickly replace with water to leach

the lye from the lye-treated fruit. Change the water several times during a 24- to 30-hour period. Avoid undue aeration and exposure of the fruit to air, for undesirable darkening of the color of the olives will result. Avoid too prolonged washing, for such practice may result in grayish-colored olives.

7. Barrel the fruit in 50-gallon oak barrels, handling the fruit rapidly and covering with salt brine as soon as possible to avoid undesirable color changes in the olives. The Queen size fruit (Sevillano or Barouni varieties) should be covered with a light salt brine (about 5 to 6 per cent salt or about 20° to 25° salometer) to avoid "salt shrivel." The brine covering the Queen olives is then increased by small additions of salt from day to day as rapidly as possible to a final concentration of 6.5 to about 8.0 per cent salt; that is, 26° to 32° salometer.

The Manzanillo and Mission varieties, not being subject to "salt shrivel," may be covered immediately with brine containing 10 to 15 per cent salt. Such brines should stabilize at about 6.5 to 8.0 per cent salt.

8. After the barrels have been filled with olives, reheaded, and filled with brine they should be rolled into the fermentation yard and placed on wooden skids to protect them from direct contact with the ground and undue exposure to moisture.

9. The barrels must be kept full of brine at all times. During the period of active fermentation when gas formation causes excessive foaming and frothing, care must be taken to replace the brine lost. Later, when gas production is not so violent, the bungs should be seated firmly enough to exclude air and thus keep film-yeast growth at a minimum. All brine lost by evaporation or expansion of the brine on hot days must be constantly replaced.

10. Supplementary sugar should be added to the brines of those olives which are lacking in fermentable material. Sugar is commonly added at the rate of 2 pounds of commercial corn sugar (such as Cere-lose) per barrel of olives. More sugar should be added to the brines of Manzanillo and Mission olives than to the Sevillano or Barouni varieties. Sugar should not be added until the fermentation has been under way for some time. The period may vary from 1 to 4 weeks, according to the time it takes the desirable lactic acid bacteria to increase sufficiently to dominate the undesirable spoilage bacteria or yeasts; otherwise spoilage of the fruit or unnecessary loss of sugar may ensue.

11. The acidity of the pickled olive brines should have a pH value of 3.8 to 4.0 and the titratable acidity should be 0.7 to 1.0 grams as lactic acid per 100 milliliters of brine before storing or shipping the fruit. This is necessary to prevent spoilage.

In conclusion, it should be stressed that the inexperienced pickler will meet with many practical difficulties in pickling green olives, but his technique will improve with experience. It is advisable that trained laboratory personnel be made responsible for accurate control of the pickling process.

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